

Agenda for non-proliferation

Governments' confusion about the future of Europe should not blind them to the need that a new regime to prevent the spread of nuclear weapons must be devised in the next five years. That is not long.

WHAT will recent events in Eastern Europe mean for the spread of nuclear weapons? While governments and the politicians of which they are composed grope for answers to the huge questions thrown up — how to distinguish self-determination from nationalism, how much investment will blunt the edge of impoverishment so as to make freedom seem worthwhile and where, in any case, might it come from? — there is a danger that less urgent, but no less important, questions will be put aside. Will, for example, new military relationships allow, even encourage, the spread of nuclear weapons? There is a sense in which, on the issue of proliferation, events in the past few months have put the clock back 30 years.

Then, many peaceful states, not to mention others more bellicose, seriously believed that nuclear weapons might be national necessities. Canada, Sweden and Switzerland, for example, encouraged by the examples of the Soviet Union and, more relevantly, of Britain and France, flirted with the notion that nuclear weapons might be a necessary safeguard of neutrality. None of them thought of becoming a superpower; it would suffice, the calculation went, to be able to inflict on an aggressor damage commensurate with their value as military prizes — the doctrine of the French General Gallois that justified French nuclear weapons. In the event, these candidate nuclear powers were dissuaded not by technical difficulties, but by political and military considerations. In Sweden, for example, it was crucial that Swedish nuclear weapons would engender increased Soviet pressure on Finland, still miraculously independent.

Will similar considerations apply in the years ahead, not just to states now in being, but to those that may yet emerge? Israel, India and Pakistan, North and possibly South Korea, are well-known black sheep. Will it now be necessary to accommodate, say, Armenia and Azerbaijan in the largely successful non-proliferation regime? And how will that be done? The considerations that weighed with the smaller powers in the early 1960s stemmed from calculations of countervailing responses within a stable political framework. It asks a lot of fate that the same should continue to be true when the framework is being rebuilt.

Institutional restraints on proliferation, on which the Soviet Union and the United States have hitherto made common cause, consist of two recognizable parts. There is the Non-Proliferation Treaty (NPT), in force since 1970,

and the agreement among the governments of nuclear suppliers that they will not supply equipment destined for the manufacture of fissile material. The treaty is a bargain between nuclear and non-nuclear powers, by which the former offered technical assistance with civil nuclear energy and promised to pursue negotiation "in good faith" on strategic arms. Neither promise has been fully kept. Technical assistance may have been less than expected for economic reasons, but the suppliers' agreement has irked many non-nuclear states, while serious negotiations on strategic arms have come to life only in the past few years. Yet the NPT requires a formal decision in 1995 on its continuation thereafter. Now is the time to begin preparing for that meeting.

The only certainty is that, by the renewal of the NPT in 1995, there will be more independently minded countries than for the past 40 years; Eastern Europe will provide seven of them, for example, each a potential nuclear power. The uncertainties are, by contrast, several. What, for example, is China's role, both as a putative superpower in its own right and as a friend of Pakistan in the endless tension with India (like Israel, a crypto-nuclear power since its nuclear explosion in 1974)? What is the continuing role of British and French nuclear weapons when the main strategic threat to Europe seems likely to be attenuated? On the face of things, their justification is attenuated, but the suspicion that the United States will not always risk its own destruction to fend off a threat to Western Europe will be increased. Even so, neither Britain nor France will be able to keep their weapons without the consent of the rest of Western Europe, however that region is in future defined.

Meanwhile, there is another historical parallel to be drawn. By 1995, the clock is likely to have been turned even further back than 30 years, but with the difference that those wishing to manufacture nuclear weapons will

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no longer have to unlock secrets previously inaccessible. The extreme version of the Gallois doctrine, from time to time echoed by China (although not recently), that stability is best assured if every state is a military nuclear power, is unlikely to command support, but there are likely to be many governments itching to set off on the nuclear road. If the NPT was a bargain between nuclear and non-nuclear states, it will have to be remade. So why not dust off and redraft the US plan for the control of nuclear weapons put to the United Nations in 1946? The underlying theme, that sources of uranium should be owned internationally, predictably came to nothing then, but administrative control of nuclear materials must be a more reliable way of spotting illicit diversions, especially if, by then, the prospect of climatic change makes the resurgence of civil nuclear power essential. □

One boycott down

Changed circumstances in South Africa will soon compel changed attitudes elsewhere than in Pretoria.

PRESIDENT F. W. De Klerk appears to have surprised his critics, not to mention his friends in South Africa, by his announcement last week that the African National Congress is to be legitimized again. In many ways as important, other political parties are no longer banned, while censorship and the death penalty are abolished. These are brave, if dangerous, steps forward. The intention is that there should be serious negotiations leading to the abolition of *apartheid* and a new constitution within five years. The process will begin when Mr Nelson Mandela, imprisoned for a quarter of a century, is released.

Two cautionary lessons must be drawn from this sequence of events, one of which is that even the most tyrannical regimes run serious risks in throwing their political opponents into goal. An earlier Soviet government discovered that by packing off the late Andrei Sakharov to Gorky. De Klerk has learned what his predecessor might have guessed, that political prisoners supported by the international community can write their own conditions for release. The second is that the abolition of *apartheid* is not simply a legislative matter, although a flood of legislation will be needed. (The South African government would be well advised to begin with a bill of rights, letting the courts shoulder the main burden of its work by striking down the practice of *apartheid*.) The success of this ambitious programme will hang on the degree to which the white minority (a third of the total) is willing to anticipate the goals now spelled out.

In that process, the international community is also engaged, both commercially and intellectually. An article on page 505 of this issue, for example, argues that ambitions in the past several years to enforce a blanket academic boycott on South Africa are mistaken (which this journal has always held), and that other forms of external pressure are likely to be more effective (and

more just). But now the question is when these and other implied sanctions against South African institutions should, in any case, be lifted. Most people will say, "but not just yet". But what happens when Mandela is released? Then those who have argued for an academic boycott must logically turn their arguments upside down. If, when there was too little sign of change in South Africa, boycott could be held justifiable, do not the changed circumstances make free intellectual exchange both seemly and constructive? □

Biotechnology eclipsed?

Genentech's rescue by Roche should raise questions about the procedures for approving new drugs.

WHATEVER has happened to biotechnology? Last week's arrangement between Hoffman-La Roche, the Swiss pharmaceutical manufacturer, and Genentech of California, is a surprise, but not a big surprise (see page 495). Like Biogen, the victim of earlier and deeper misfortunes, but also the other conspicuous star among the constellation of ambitious new biotechnology companies ten years ago, Genentech has learned the hard way how great is the cost of bringing new drugs to the market. For practical purposes, the company has two marketable products to show for ten years' hard work. During that time, it has built up an enviable reputation for the breadth of its laboratory investigations and for its readiness to devote resources to the investigation of common problems — and to publish the results. But, plainly, reputation is not simply converted into dollars; two years ago, Genentech's tissue plasminogen activator was kept waiting for six months by the Food and Drug Administration while a scientific opinion was supported by the prescribed data.

While some in the United States will regard this new development as yet another offence against the stars and stripes, others of a more reflective spirit may rightly ask whether Genentech's troubles derive from the arrangements now in force for the registration of new drugs. It is proper and prudent that synthetic chemicals believed to be effective against this or that disease should be put through the now-familiar system of toxicity and efficacy tests in animals and human beings. But is the full and expensive apparatus of these enquiries necessary when the materials on trial are intended to be identical with those produced naturally in normal human beings? So far, thanks to the measures taken to avoid public anxiety concerning recombinant-DNA techniques in the 1970s, the products of genetic manipulation have been put through the same rigorous and probably over-rigorous mill. How many more Genentechs will have to seek safety in the arms of bigger companies before this practice is recognized as the modern equivalent of the quaint British practice of sending a man with a red flag in front of the early railway locomotives? □

HERA magnets may need to be upgraded

■ 'Eddy currents' spoil magnetic field

■ Upgrade to cost \$300 million

Munich & Washington

UNFORESEEN problems with the superconducting magnets in the nearly completed HERA (hadron electron ring *anlage*) electron-proton collider in Hamburg, West Germany, may force a costly eleventh-hour upgrade. The technical difficulties are similar to those encountered on prototype magnets for the US Superconducting Super Collider (SSC), which last month led to a costly redesign. But HERA physicists are still optimistic that they can solve the problems with minor, and relatively inexpensive, modifications.

For both HERA and the SSC, the problem begins with the fact that protons are fed by a lower energy accelerator — the injector — into the main ring at an energy much lower than the final energy at which collisions are to take place. Designers of both machines have found that at the injection energy, when only a small current is passed through the superconducting main ring magnets, unexpectedly large 'eddy currents' spoil the magnetic field quality and send the protons crashing into the beam-pipe walls.

Both HERA and the SSC were initially planned with the final energy a factor of twenty higher than the injection energy.

HERA accelerates protons from 40 GeV up to 820 GeV, and the SSC was to take protons from its injector at 1,000 GeV, or 1 TeV, to a collision energy of 20 TeV.

The eddy current problem was discovered in the SSC magnets in the prototyping phase, and SSC officials have decided to double the injector energy to 2 TeV so that the main ring magnets do not need to be run at such low currents. The upgrade, which will cost nearly \$300 million, has led to concern that the project's cost might pass its 'political threshold' and lose it congressional support (see *Nature* 343, 103; 1990).

Early tests on the superconducting magnets at HERA have revealed the same problem. The difficulty would be avoided if the protons were injected at 80 to 100 GeV, making the injection to final energy ratio about 10, as in the redesigned SSC. But in HERA's current design, the injector is an existing machine known as PETRA, and redesigning it would mean throwing PETRA away and building a wholly new injector. The cost of such an overhaul would be about DM30 million, (\$18 million) according to HERA research director Paul Söding. Nearly DM1,000

million has already been spent on the machine.

Nevertheless, physicists at HERA are still optimistic that they will be able to avoid the SSC's problems and are now trying to build additional magnets that will counteract the eddies.

Unfortunately, the task is not simple. The eddies vary irregularly with time, making it difficult to build compensating magnets.

The problem is made worse because HERA includes ostensibly identical magnets made by separate teams in Italy and West Germany. The magnitude and behaviour of the eddies depends strongly on the internal structure of the superconducting cable, and it turns out that magnets made in Italy behave differently from those made in West Germany. Two different strategies for compensation have had to be developed, and Italian magnets and West German magnets will be bunched in alternating octants around the ring. HERA officials will make no decision on an injector upgrade until the main ring is finished, later this year, and are hoping that some combination of these small alterations will dispose of the eddy problem.

US researchers familiar with the project are sceptical that the West German scientists will solve a problem that stumped teams of SSC planners. "It's clear that they'll get some particles to go around, but the question is how many", says SSC physicist Roger Koons. One of the key factors in a collider is the rate at which collisions occur, which depends on the beam luminosity. An accelerator that directs particles onto a fixed target can overcome a low luminosity by running for longer times, but for colliders such as HERA and the SSC, a certain minimum luminosity is essential if any collisions at all are to be produced.

SSC researchers say they are watching the developments at HERA closely. One lesson the US researchers have already learned is not to mix superconducting cable from different sources. Because of HERA's difficulties, the SSC will only use cable either manufactured or supervised by a single vendor.

"If you don't understand the physics of superconducting cable, the next best thing is to make it all exactly the same", says Paul Mantsch, a physicist at the Fermi National Laboratory where the SSC magnets are being designed.

**Steven Dickman &
G. Christopher Anderson**

INTERNATIONAL BIOTECHNOLOGY —

Swiss company takes a 60 per cent stake in Genentech

Washington

LAST Friday's announcement that the Swiss-based multinational health-care conglomerate, Roche Holdings Ltd, is to spend \$2,100 million for a 60 per cent stake in Genentech, Inc., the largest US biotechnology company, took Wall Street and the biotechnology world by surprise. The proposed 'merger' was generally well received and sent Genentech stock soaring by almost \$8 a share by the end of Friday. The deal is expected to give Genentech the financial depth to take new products from development through the drug approval process to commercial fruition. But coming at a time of increasing concern over foreign investment in US industry, the agreement raises the question of whether smaller biotechnology companies can hope to remain independent and still realize their long-term potential.

Under the agreement, which is subject to approval by stockholders in April, Roche will purchase half of Genentech's existing shares at \$36 each — 65 per cent above last Thursday's closing price.

Roche will also spend a further \$492 million in capital to buy new shares, bringing its total ownership to 60 per cent. Despite its majority equity interest, Roche will have only two members on the 13-member Genentech board, allowing Genentech to continue as an independent, publicly traded company with control over its research programme. Genentech has been soliciting such a merger since the summer.

The deal comes at a crucial time for the San-Francisco-based Genentech, whose 1989 revenues were \$400 million. Apart from the approval of additional uses for existing drugs, the company will not have any significant new products on the market for a few years.

Gamma interferon, which initially will be used to treat a rare immune-related disorder, and a CD4-based AIDS drug are in the late stages of clinical trials. Genentech therefore needs to increase sales of the two products currently on the market, the blood clot-dissolving drug Activase (tissue plasminogen activator, TPA) and human growth hormone.

Sales of TPA failed to live up to expectations after its approval in 1987, and stock prices fell to below \$15 a share in 1988 from a peak of more than \$60 in 1987. The high cost of TPA, which at \$2,200 a dose was ten times that of a rival drug Streptokinase, was an obstacle to its widespread use. Recently, TPA has been challenged by SmithKline Beecham's Eminase, which is more easily administered and costs \$1,700 a dose.

Diane Gershon

Counterpart to 'Frontiers'

Tokyo

JAPAN's Ministry of International Trade and Industry (MITI) is about to launch a sequel to the Human Frontiers science programme. But unlike Frontiers, which is aimed at basic research on the brain, the new project is aimed at the development of manufacturing technology, an area in which Japan is a world leader.

The Intelligent Manufacturing System (IMS) project is the brainchild of a committee of academics, industrialists and MITI officials headed by Hiroyuki Yoshikawa, dean of the faculty of engineering at Tokyo University. The committee, established last year, proposes the establishment of an international research centre in the United States or Europe with funding of about \$1,000 million over ten years. In the committee's proposal, which was explained to government officials of the United States, Europe and Australia at a meeting on 19 January, the Japanese government and private sector will provide about 60 per cent of the funds and other countries the rest.

The institute will develop fully automated manufacturing systems, including product design, intelligent robots for product manufacture and automated systems for retailing and distribution. An important element will be to develop standardized systems that can be inter-linked and used anywhere in the world.

Research at the proposed institute will be divided into about 100 projects over the ten-year period, each with budgets of about ¥1,000-¥1,500 million (\$7-\$10 million). MITI has won the unusually large sum of ¥110 million (\$700,000) in preparatory funds for the project in the budget allocations for fiscal year 1990 set by the Ministry of Finance at the end of last year.

Yuji Furukawa of the department of mechanical engineering at Tokyo Metropolitan University, one of the project organizers, says that about a hundred 'core member' companies will donate ¥12 million (\$85,000) each to finance the conceptual design of IMS. Already 50 companies, including Toshiba, FANUC (one of the world's leading manufacturers of machine tools and robots), Kawasaki Heavy Industries and "several big US companies", have formally or informally agreed to join, according to Kunihiko Umehara of the Industrial Machinery Division of MITI. And last week the Michigan-based Society of Manufacturing Engineers agreed in discussions with Furukawa to act as a 'window' for the project in the United States.

Organizers of the project are asking for project proposals from companies, universities and research organizations in

Japan and overseas to be submitted by 30 June at the latest. If all goes according to plan, an international committee of academics drawn from the United States, Europe, Japan and possibly other countries will assess the proposals and draw up firmer plans for the project at a meeting on 3 and 4 June in time for MITI to submit a budget request for fiscal 1991 in late August.

The project is in many ways reminiscent of Frontiers but has important differences. IMS, like Frontiers, is being promoted as a way of 'paying back Japan's debt to the Western world' at a time of massive trade deficits with the West. And when first proposed by MITI, Frontiers, like IMS, was put forward as a means of developing intelligent robots. But this goal was quietly dropped by the academics from Japan and Western nations who moulded Frontiers into its final form. For IMS, it is academics who are proposing robot development.

Umehara says the IMS research institute will probably be set up in the form of a foundation, like Frontiers. But, unlike Frontiers, which took four years of protracted discussions to launch and shrank from an initial proposal of \$350 million a year to \$20 million, Umehara expects IMS to get under way next year with a level of funding close to that proposed.

A foreign government official who

attended the briefing on 19 January says that MITI has obviously learned a lot from Frontiers. This time, pamphlets describing the project in English are clear and lucid. MITI has avoided involvement of other ministries and agencies which delayed the launch of Frontiers.

Yoshikawa and Furukawa have done considerable groundwork, visiting government officials, universities and research institutes in the United States and Europe to explain the project and win support before its announcement. And the topics for research are in areas where Japan has considerable expertise, making IMS less likely to be interpreted as an attempt to pick the brains of Western scientists.

Nevertheless, the London *Financial Times* newspaper has suggested that the project's aim to standardize manufacturing technology might make it easier to duplicate Japanese factories outside Japan and that Japan is lagging in the development of computer software for use on the shop floor. But Furukawa says that IMS has nothing to do with the movement of Japanese factories overseas. The aim of IMS is to transfer Japanese expertise in manufacturing to the West and to develop new fully automated and standardized technologies for the "benefit of all mankind". And Umehara says that foreign government officials, academics and members of industry have given a generally "warm response" to IMS in contrast to the "chilly reception" that first greeted Frontiers.

David Swinbanks

PEST CONTROL

Double blow for blowflies in Australia

Sydney

AUSTRALIAN scientists are to test a new method of controlling the blowfly (*Lucilia cuprina*), an agricultural pest of sheep which costs farmers A\$200 million a year, through the release of blowflies that have been genetically manipulated in a unique way.

Scientists at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) will release 700 million blowflies during the summers of 1990 and 1991 on the remote Furneaux group of islands north of Tasmania. Each male fly will deliver a double genetic blow. A sex-linked translocation will cause a high frequency of sterility in the male line while several other genetic mutations will bring about blindness in the female line.

According to Rod Mahon, senior research scientist within the Division of Entomology at the CSIRO, the Furneaux experiment is the first use of genetic manipulation to suppress an insect population. "Ongoing suppression is achieved as a result of the blindness mutation and sterility", he says, "the sons and daughters

continue to carry this mutation unlike other suppression systems in which only sterile males are released." In the ongoing screw-worm eradication programme in Texas and Florida the continuing release of infertile males is necessary. "If a release is missed, suppression is lost", says Mahon.

In a small trial using the genetically manipulated blowflies, mutations were still present in the population nine months after the release of males had ended. The blowflies are released from the air so a reasonably high density of sheep per hectare is needed to make the system cost-effective. "We feel it is possible to totally eradicate the sheep blowfly from the island state of Tasmania but on the mainland a strategy of continuing suppression, in selected high risk areas, may be more cost effective than attempting eradication", says Mahon.

The *Lucilia* blowfly was introduced to Australia, probably from South Africa or India. It is, according to Mahon, extremely well adapted to living on live sheep and can rapidly develop resistance to insecticide as it undergoes 6-10 generations a year.

Tania Ewing

EAST GERMANY

West Germans help the East

Bonn

In an attempt to stem the tide of East German academics flooding westwards, the West German research agency DFG (Deutsche Forschungsgemeinschaft) announced last week that it would allocate new money to support collaborative projects between East and West Germany. This will be the first time since 1945 that DFG has supported projects in East Germany.

Grant applications must be submitted jointly by a West and an East German partner, and will be evaluated through the same peer-review procedure that DFG normally uses. DFG has asked the government for DM5–6 million (\$3–\$3.6 million) in additional funds in 1990 to finance the programme, estimating that about 100 new projects could then begin. But DFG president Hubert Markl said the plan "will go ahead even if we don't get the money", and declared "We must respond now to this situation," even if it means making sacrifices.

President Heinz Bethge of the Akademie Leopoldina in Halle welcomed the DFG programme as a way to help East Germany begin rebuilding a science infrastructure weakened by years of Communist Party favouritism. The Akademie Leopoldina, which was founded in 1652, eight years before the Royal Society of London, is one of the few organizations that remained relatively free of Communist influence.

Bethge said that the money was needed to "wake the frustrated generation" of researchers, some of them now in their fifties, who were kept down by the system of party privilege, said Bethge. "Giving these people courage is our only hope" for rebuilding East German science, he said.

The West German Research Ministry (BMFT) is likely to follow DFG with an East German cooperative programme on a much larger scale.

Preliminary reports say BMFT will request an additional DM40 million in the 1990 budget for projects in East Germany. In addition, permission is expected to be granted by the Finance Ministry for

BMFT to redirect DM40 million in the current 1990 budget for East German projects. The money will be awarded in areas of applied science such as nuclear reactor safety, coal technology, telecommunications, microelectronics, materials research, and in support for 'technology-orientated companies'. Like DFG, BMFT put the emphasis on collaboration. "We're not the 'GDR Development Ministry'," said a BMFT spokesman. "We expect at least minimal input from East Germany in all these areas." But he hinted that as much as DM1,000 million could be directed through other ministries' budgets for investment in East Germany. That money might be used for projects such as a modernization of East Germany's inefficient and heavily polluting coal-fired power plants.

Despite the enthusiasm, there is a feeling of tentativeness at both DFG and BMFT about the future of these programmes. Both assume that the money will be invested in a sovereign East German state, but there is a growing feeling that East Germany may not last long. One observer gave independent East Germany a "half-life" of six months.

East Germans, researchers included, have forced politicians to act on reunification by simply heading to the West. In an admittedly unscientific sample, Hermann Scheuer of the Large Research Establishments (AGF) in Bonn reported that between 9 November and mid-January, nearly one East German researcher per day was contacting AGF from West Germany, asking about job opportunities.

Markl emphasized that DFG wants to encourage researchers to remain in East Germany. "If they come to the West", he said, "they will have to compete for funding with all of the groups already established here." But he denied that standards would be lowered for the joint applications.

Markl admitted that a few million deutsche mark would not overcome the entrenched Communist power structure in the scientific establishment in East Germany. "We're not so naive to think

Appeal for help

Bonn

BENNO PARTHIER, the president-elect of the Akademie Leopoldina in Halle, East Germany (see left), appealed to university professors in the West to come to East Germany to teach. "We desperately need the kind of help that only professors trained in the West can give", implored Parthier; "we would welcome visitors from the United Kingdom, France or anywhere else who would like to come and share their expertise with our students." Parthier said he was sure that East German students understood enough English to listen to lectures in that language as well as in German. President Hubert Markl of the DFG added his voice to Parthier's appeal, asking West Germans in particular to help. "I'm convinced that they would return from the experience personally enriched," he said.

As there is no central authority for arranging guest professorships at East German universities, those who are interested should get in touch with the universities directly or with the Deutsche Akademie der Naturforscher Leopoldina, August-Bebel-Strasse 50a, DDR-4010 Halle, German Democratic Republic, telephone 37-46-25014. Steven Dickman

that the people who will get some of the money are not the same" as the ones who set up the old structure, he said. But by directing the programme toward individual researchers instead of institutions, DFG hopes to encourage a new structure.

Bethge agreed enthusiastically with Markl's logic. "Eighty per cent of university professors were in the Party", he said, which helped contribute to a "slide" in scientific standards. Sixty per cent of these people should just be "thrown out", said Bethge, but he admitted that this kind of "denazification" is not possible. "All we can do is try to replace them slowly with better people" selected from the ranks of the frustrated, he said.

Biochemist Benno Parthier warned that even with help from the West there would be difficult days ahead for East German science. The decline in scientific standards over the past ten years matches the decline in the economy revealed after the Communist government fell. The increase in patents reported by the academy in 1989, 10 per cent more than in the previous year, is a "meaningless statistic," said Parthier: the patents are mostly worthless.

The science minister of the interim East German government, Peter-Klaus Budig, offered only disappointment to basic researchers at a recent plenary session of the Academy of Sciences, Parthier reported. Budig's top priorities for science were "energy policy, transport problems and communication problems"; biology was listed tenth. Steven Dickman

INDIA

Sex selection continues in Maharashtra

Bangalore

LEGISLATIVE attempts to prevent the selective abortion of female fetuses in India's western state of Maharashtra have failed, according to an Indian pressure group, the Forum against Sex Determination and Sex Pre-selection. In 1988, Maharashtra enacted India's strictest legislation on the issue, banning the use of amniocentesis for fetal sex determination. But the political will

needed to implement the act has failed to materialize and nationwide legislation on the misuse of medical techniques in sex-selective abortion is still lacking.

The Maharashtra act lays down detailed procedures for complaints. But in a recent test case, the forum found it was impossible to get the government to take action against a clinic that advertised sex determination services. Radhakrishna Rao

PET for dementia drug test

Tokyo

A GROUP of seven Japanese companies is expected to launch a government-backed project in April that will use Positron Emission Tomography (PET) of the brain to develop and test new drugs for the treatment of senile dementia. The PET project, which has a particular urgency because of Japan's ageing population, will be supported through the Ministry of Health and Welfare (MHW) as part of a new scheme designed to promote industrial research.

The money for this project comes from a fund originally set aside to provide financial relief to patients who had suffered adverse reactions from drugs. But by 1987 this fund had acquired unused assets of more than ¥3,000 million (more than \$20 million), and the MHW decided to plough some of the money back into research and development, renaming the organization the Drug Fund for Adverse Reaction Relief and Research Promotion.

Each year two new projects, chosen from applications that must involve two or more companies, are initiated. Last year 13 groups applied, and two of the proposed

sals involved the use of PET scanners. Successful applicants establish a joint-venture company and the fund provides up to 70 per cent of required research and development funds (typically ¥4,000–¥5,000 million (\$29–\$36 million) over a period of seven years by acquiring stock in the company. After seven years the company can be continued with private funding or dissolved. Intellectual property rights arising out of the research belong to the company.

The successful PET project application comes from a group of four pharmaceutical companies (Takeda Chemical Industries Ltd, Fujisawa Pharmaceutical Co., Yoshitomi Pharmaceutical Industries Ltd and Tanabe Seiyaku Co., Ltd), Wako Pure Chemical Industries, Ltd (a subsidiary of Takeda that deals in diagnostics), Nihon Medifix (a company owned by Hoffmann-La Roche that sells radioisotopes) and Sumitomo Heavy Industries.

Katsura Morita, executive vice-president of Takeda, says that the main purpose of the project will be to discover, appraise and develop new anti-dementia drugs. Senile dementia is a matter of great concern to the Ministry of Health and Welfare because of the rapid ageing of the Japanese society. The group will study the two main types of senile dementia, which are caused by blood vessel obstruction in the brain and by deficiency in neurotransmission. PET will be used to monitor the brain activity under the influence of new drugs.

Takeda has considerable expertise in the development of such drugs and in the rapid synthesis of short-lived radioisotope-labelled materials injected into patients for PET scanning. Sumitomo manufactures mini-cyclotrons that will be used to produce the radioisotopes. One difficulty that the group intends to overcome will be the comparatively poor resolution, about 3–5 mm, of current PET scanners. But Morita is confident that this barrier can be overcome by using suitable image-processing techniques.

Formal announcement of the new project by the ministry is not expected until the end of this month. But the group is already negotiating with a hospital to support the project. It will take about a year to a year and half to put together all the hardware, but Toyokazu Kishi of Takeda says that the group hopes to begin experiments with 'cold' (unlabelled) drugs in April. One of the first drugs the group will look at is Spiperone, a neurotropic and antipsychotic drug marketed by Janssen. Spiperone has a strong affinity to dopamine receptors, making it and its derivatives especially useful for PET brain studies, according to Morita.

David Swinbanks

Increase or reshuffle?

London

THE UK government's plans for public spending for the three years to 1992–93, released last week, contain an increase for higher education of 10 per cent in 1990–91 from 1989–90, with funding remaining at about this level for the following two years. But some of this increase has to be offset against increased costs: the student population is expected to grow by 10 per cent over the same period.

The planning figures reflect the government's desire to move away from meeting university teaching costs through centrally provided grants towards a system of fees. The Universities Funding Council (UFC) and the Polytechnic and Colleges Funding Council (PCFC) are faced with shrinking budgets because, to link teaching provision more closely to student numbers, some UFC and PCFC money is to be transferred to local education authorities (LEAs), whence it will go to education institutions as fees accompanying each LEA-supported student. From next autumn, these fees are to be increased from £607 per student per year, to £1675.

The shift in UFC and PCFC money to LEAs is "financially neutral", in terms of overall higher education spending, according to the Department of Education and Science. But there is concern that not enough money from the funding councils' budgets will be left for faculty pay settlements, once funds earmarked for building repairs and restructuring programmes have been removed.

The UFC last week announced a small concession to opponents of its competitive bidding system for student places (see *Nature* 342, 843; 1989), introduced by the government to accompany the shift towards fees. Universities must state how many students they wish to teach in each subject from 1991–92, and offer a price for teaching costs per student. A list of guide prices, released early in January, was intended to set the maximum teaching costs.

But the UFC has now said that "in exceptional circumstances", it will consider offers above the guide price. It is not yet clear what these circumstances would be, but Sir Peter Swinnerton-Dyer, chief executive of UFC, expects "very few bids above the guide prices to be justifiable".

The government public expenditure plans also include more than £560 million over three years for the introduction of its controversial student 'top-up loans' scheme. From next autumn, the government intends to freeze the student maintenance grant at its present cash level, providing further finance through loans. The government remains committed to the scheme, despite the major banks' decision, mindful of student custom, not to administer the proposed student loans company.

Peter Aldhous

'Wellness Letter' tops subscription stakes

San Francisco

THE Wellness Letter, a monthly newsletter of health maintenance published by the School of Public Health of the University of California at Berkeley, has now attracted one million subscribers, making it the most widely circulated newsletter in the United States, the university reported. Founded in 1984 with 28,000 initial subscribers, the \$20-a-month publication tries to make sense of the sometimes contradictory findings of health researchers on such issues as the effects of caffeine, and recommends ways for people to improve their health.

The eight-page February issue includes articles about decaffeinated coffee, a discussion of the 'empty nest syndrome' suffered by parents who are left alone for the first time in years when their children grow up, and a 'buying guide' section devoted to toothpastes: flossing remains the best tartar-control method, readers are cautioned, despite the claims of some new 'tartar-control' toothpastes.

The newsletter is distributed in Canada, West Germany (as the 'Gesundheit Report'), and Australia as well as the United States. Royalties are being used to sponsor 'Wellness Fellowships' for graduate students, guest speakers and visiting scholars at the School of Public Health.

Robert Buder

BRITISH SCIENCE

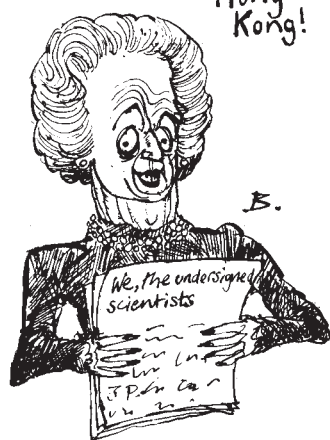
Home thoughts from abroad

London

A PETITION demanding increased government investment in science, and signed by more than 1,600 British 'brain drain' scientists working abroad, was presented yesterday (7 February) to Prime Minister Margaret Thatcher. The petition is the work of British Scientists Abroad (BSA), an academic pressure group set up last year by young British scientists working in California. More than half the signatories have faculty-level positions; 24 are fellows of the Royal Society and over 100 are heads of departments.

Poor career prospects, inadequate research equipment and the abolition of tenure are driving British scientists abroad, according to the BSA, which says that as a proportion of Gross Domestic Product,

It doesn't matter: we're getting plenty more from Hong-Kong!



science spending in the United Kingdom is less than in comparable countries such as France and West Germany.

Matthew Freeman, a postdoctoral molecular biologist at Berkeley, California, who helped to organize the petition, says that interest was spurred by a letter from science minister Robert Jackson to the Committee of Vice-Chancellors and Principals (CVCP) in July 1989. Jackson dismissed the British brain-drain scare as a rumour, and challenged the CVCP to come up with hard evidence.

CVCP and the lecturers' union, the Association of University Teachers, have since commissioned a study into recruitment and retention of academic staff.

Poor academic salaries in Britain have contributed to the brain drain, according to Michael Duff, professor of physics at Texas A&M University since 1988, who flew to London with a number of other scientists to present the petition. "Competent British academics can easily triple or quadruple their salaries by moving to the United States", he says, and US universities view Britain as a "happy hunting ground" for recruitment. Peter Aldhous

SPACE STATION

Rescheduling reconsidered

Washington

REACTING to criticism from its European partners, the US National Aeronautics and Space Administration (NASA) has begun a new and comprehensive review of its construction schedule for Space Station Freedom. Among the changes now being considered are launching Columbus, the \$4,500-million experimental module from the European Space Agency (ESA), nearly a year earlier than planned, increasing the size of the space station's solar panels to provide more electrical power and including a Canadian robotic arm earlier in the construction process.

European, Japanese and Canadian space officials have been unhappy with NASA in recent months for making significant changes in the project's schedule without consulting them. Last autumn, NASA 'rephased' the space station after Congress cut nearly \$400 million from the project's 1990 budget. The foreign partners complained that in the rephased plan most of the international components of the space station were delayed, but most of the US components remained on schedule. In congressional testimony last week, officials from ESA and the Japanese National Space Development Agency (NASDA) said they found out about the NASA schedule review — known as the 'Langley Exercise' — only after it was under way, too late to change its outcome.

ESA director-general Reimar Lüst told the House of Representatives Committee on Science, Space, and Technology that he and other ESA officials met with NASA administrator Richard Truly late last year to register their unhappiness with the Langley Exercise. The unilateral change of schedule "departed radically from both the letter and the spirit" of the memorandum of understanding between the partners, he said. ESA officials told Truly at the time that their financial support for the new plan would not be assured until European concerns had been addressed.

Last week NASA officials sent ESA and NASDA the initial results of a 'complete systems engineering study' they had ordered to find rescheduling options that the international partners might find more acceptable.

The principal change would be to launch the Columbus module in the third quarter of 1997, instead of in June 1998 as the Langley review had recommended. Lüst told the House committee that advancing the launch date by nearly a year could save ESA between \$200 and \$300 million. But in a letter to ESA, NASA warned that bringing Columbus up earlier would leave the rest of the space station with only enough power to "stay alive" until the full solar panel array was installed. "Signifi-

cant utilization" by the United States, NASA said, would be delayed by at least eight months, something the US scientific community might find "unacceptable". Another review later this month will consider the possibility of installing larger solar panels on the space station, an upgrade that could give users 6 kW (20 per cent) more power and perhaps ease concern both at home and abroad. But NASA warns that the enlargement is likely to be expensive, and could increase atmospheric drag enough to affect the station's orbit adversely.

NASA is also considering the practicality of bringing a Canadian robotic arm earlier in the construction sequence. It is currently scheduled for the fourth shuttle flight, Canadian officials say that their country's participation in the most visible part of the construction is politically important in securing support at home.

Officials from the partner nations reacted favourably last week to NASA's efforts to find a mutually agreeable compromise. "The new options give an indication that NASA is willing to take our concerns into account, which is very welcome. Up to now we have been brushed off", says Derek Deil, head of ESA's Columbus liaison office.

G. Christopher Anderson

PALAEOPHILATELY

'Dinosaur' birthday to be celebrated

London

THE 150th anniversary of the invention of the term 'dinosaur' will be officially marked in 1991 by a commemorative set of dinosaur stamps, the UK Post Office announced this week. The word was coined at the 1841 meeting of the British Association for the Advancement of Science (BAAS) in Plymouth, where the anatomist Sir Richard Owen described the flesh-eating reptile *Megalosaurus*, the herbivorous *Iguanodon* and the armoured *Hylaeosaurus*. The BAAS is due to hold its 1991 meeting in Plymouth, and Owen's etymological invention will be part of that year's celebrations.

Palaeontologist Beverly Halstead of Imperial College, London, will be president of the geology section of the BAAS in 1991, and has been campaigning for the special stamp issue. Part of the appeal will be that the first dinosaurs to be described were found in the British Isles.

The dinosaurs that will be depicted have not yet been chosen by the Post Office, but spokesman Barry Robinson confirmed that there was a "firm intention" to issue them. Suitable candidates would include the species mentioned above. Henry Gee

EPO licensing talks break down

Washington

CROSS-licensing negotiations over the US rights to erythropoietin (EPO), a kidney cell glycoprotein that stimulates red-blood-cell production, broke down last week, leading Genetics Institute and its licensee, Chugai Pharmaceuticals of Japan, to go on the legal offensive against its rival, Amgen Corporation.

The battle over conflicting patent claims to EPO, whose US sales have been estimated at \$200-\$1,000 million over the next 3-5 years, has been long and tangled. Each side has claimed primacy, but last December a Boston court upheld the central claims of both companies' EPO patents, while also declaring them partially invalid and mutually infringing (see *Nature* 342, 846; 1989). This stand-off seemed to set the stage for some form of cross-licensing agreement.

But negotiations on cross-licensing failed, and Genetics Institute and Chugai Pharmaceuticals have now asked a US district court in Boston to grant a permanent injunction against Amgen from infringing on Genetics Institute's EPO patent. If granted, this would prevent Amgen from manufacturing and selling its version of EPO — Epogen — in the United States. Chugai Pharmaceuticals manufactures the Genetics Institute version of EPO, Marogen, and because it is made outside the United States and imported, its manufacture does not infringe Amgen's EPO patent.

But Marogen has not yet been approved by the US Food and Drug Administration (FDA), and recognizing that Epogen is the only EPO drug approved for sale in the United States, Genetics Institute proposes a stay of its own injunction, pending appeal of the December ruling, on the grounds that it would not be in the public interest to prevent 44,000 patients with chronic renal failure from receiving Epogen for the treatment of anaemia.

During the appeal period, Genetics Institute and Chugai Pharmaceuticals have requested that Amgen make potential 'damage payments' by depositing profits from the sale of Epogen into an escrow account.

Finally, Genetics Institute and Chugai Pharmaceuticals have asked the court to order Amgen to certify that it cannot assure future supplies of Epogen because of the patent infringement ruling handed down in December. They believe this certification would clear the way for the FDA approval of Marogen despite the fact that Epogen enjoys orphan-drug status. Under the orphan drug rules, Amgen has a seven-year monopoly on EPO products in the United States because the market for it is limited, but only if it guarantees to supply as much Epogen as is needed. The hearing is set for 14 February.

Diane Gershon

Kvant brings Soviet discipline

Washington

ONE of the symptoms, or perhaps causes, of the relative decline of science education in US high schools is the fact that an excessive interest in science or mathematics is not the way to win the approval of one's peers. In the Soviet Union, if the first US edition of *Quantum*, a translation of the Russian magazine *Kvant*, is any guide, things are different.

Quantum is full of mathematical problems, exercises in classical mechanics, and chess puzzles, all of which, apparently, Soviet teenagers feel able to discuss shamelessly in public. If *Quantum* does nothing more than make such activities socially acceptable, it will have done a great deal for US high school science.

Kvant was begun in 1970 by the remarkable Soviet mathematician A. N. Kolmogorov, and now circulates in a monthly edition of 200,000 to Soviet schools. The first US issue contains direct translations of half a dozen original Russian articles and is being printed in a run of 65,000 copies, which will go to universities, colleges and high schools throughout the country.

This first issue, and another one to appear next April, are being supported by a three-year grant from the National Science Foundation (NSF). From next autumn, the plan is to produce *Quantum*

CLIMATE CHANGE

Bush chooses caution

Washington

In a speech tainted by allegations of internal White House bickering, President George Bush opened a United Nations meeting on climate change with a warning that politics should not dictate policy-making on global warming.

Addressing the third plenary session of the UN Intergovernmental Panel on Climate Change in Washington, Bush's speech was notable for its absence of any direct mention of the greenhouse effect.

He chose to focus on general environmental issues such as conservation and protection, and emphasized the need to balance environmental concerns with economic policy, which he said "need not be contradictory". Bush acknowledged the existence of a "broad spectrum of views" on the issues, and said that more accurate models were still needed: "where politics and opinion have outpaced the science, we are working to bridge the gap", he said.

The *Washington Post* reported this week that John Sununu, Bush's chief of staff, edited out remarks designed to underline the seriousness of global warming. Sununu opposes restrictions on coal and oil burning to reduce greenhouse gas emissions and has questioned the reliability of current climate models.

G. Christopher Anderson

quarterly, with a subscription price of \$9.95, and with an increasingly number of articles from US authors. The intention in the long run, according to production editor Elisabeth Tobia, is that *Kvant* and *Quantum* should be sister publications.

There has been a positive response from those high-school teachers who have seen *Quantum*, according to Tobia, and numerous enquiries from others who have heard about it. In a preface, physicist Sheldon Glashow, one of the US editors, remarks that most of his Soviet colleagues were encouraged in their youth by *Kvant*, and many now write for it.

With the exception of a two-page feature giving unadorned accounts of some computer chess games, the articles in the first issue are devoted entirely to mathematics and physics; in one, the speed of a Viking ship in a painting is estimated from the artist's (inaccurate) rendition of the bow waves, and another describes some geometrical ideas that can be derived by folding sheets of paper. The 'problem-orientated' slant of the first *Quantum* will change, Tobia says, once the US edition begins in earnest. A balance of biology and chemistry will be included, and there will be articles on social aspects of science, environmental matters being a topical example.

David Lindley

NUCLEAR WEAPONS TECHNOLOGY

No fuel enrichment

San Francisco

A controversial plan to use lasers at the Lawrence Livermore National Laboratory (LLNL) to enrich plutonium fuel for atomic bombs has been dropped by the US Department of Energy (DOE). A plan to build a full-scale plutonium enrichment plant in Idaho using the LLNL technology has also been set aside.

Both decisions are direct consequences of the omission of funds for the Idaho project from last week's US budget announcement, said LLNL spokesman Jeff Garberson, with the Idaho plan in abeyance it seemed pointless to push ahead with full-scale technical development using the plutonium at Livermore, he said. LLNL researchers will work on the fuel-enrichment technique using a substitute non-radioactive material.

Abandoning the idea for the time being also avoids the prospect of time-consuming legal and bureaucratic entanglements. Several environmental groups have vigorously opposed both the Idaho facility and the idea of bringing additional plutonium to the Livermore site. Earlier this year, the DOE announced plans to begin an Environmental Impact Report that Garberson said would have taken at least a year to complete.

Robert Buder

BIOTECHNOLOGY

Europe falling behind

London

A GROUP of prominent European industrialists has sent out a warning that European Community (EC) policy on biotechnology is falling behind that of the United States and Japan to such an extent that Europe's future position in the biotechnology industry is in jeopardy. In a paper circulated last week, the Senior Advisory Group on Biotechnology (SAGB) says that many of the biotechnology projects being paid for by the EC are "too limited in both scope and scale to have lasting impact and give a clear European profile". More ambitious projects, such as genome mapping and the design of new molecules, are recommended. And to encourage the growth of a biotechnology industry, better patent protection and more coherent product regulation are needed.

EC policy on patent protection for genetically engineered organisms is incoherent, according to Tim Roberts of ICI, particularly in the case of plants. In the United States, by contrast, a series of test cases in the 1980s established a fairly

firm legal basis for plant patenting. Roberts believes a similar system is needed in Europe, so that the 'inventor' of, for example, a potato incorporating a fungicide-producing gene could obtain royalties when the same gene was subsequently bred into other varieties of potato. A European Commission directive, to be considered by the European Parliament in May, may give protection along these lines. But draft regulations from the Commission's Agriculture Directorate-General propose instead that patent protection should be removed for individual plant varieties once a plant breeder has satisfied the narrower criteria for commercial plant variety protection.

The SAGB paper also argues that proposed EC policy on product regulation may disadvantage the biotechnology industry. Nigel Poole, a member of the SAGB support group, says that existing product-safety legislation can and should

be applied to the products of industrial biotechnology.

What matters are the inherent characteristics of a product and its intended use, he says, regardless of whether or not it has been produced by genetic engineering. "Without a suitable framework of legislation, we cannot justify the long-term development of products", Poole says.

Two forthcoming EC directives will lay down strict rules on the contained use and deliberate release of genetically manipulated organisms (GMOs). The rules will apply to academic research projects as well as to commercial products containing GMOs. Despite the SAGB's opposition to stricter controls for biotechnology, and sympathy for the industrialists' views from some quarters within the European Commission, the directives will probably be adopted in March and implemented through national legislation. Several member states, including the United Kingdom (see *Nature* 343, 4; 1990) are already drawing up their own laws.

Peter Aldhous

ANTARCTIC RESEARCH

Four dead on Indian expedition

New Delhi

INDIA'S ninth Antarctic expedition has run into tragedy. Four members of an expedition have died and two others were incapacitated during the first week of a visit to the Weddell Sea. The Department of Ocean Development (DOD) said that the deaths were from poisoning by carbon monoxide from a diesel generator kept inside the men's sleeping quarters. An investigation has been launched.

Three of the dead were from the Geological Survey of India and the fourth was a Navy communications engineer. A request by India for assistance received a prompt response from other members of the Antarctic research community. The Soviet Union has offered to help in transporting the bodies to New Delhi, and one of the sick expedition members, suffering from a duodenal ulcer, was flown in a West German plane to the South Pole, where a US aircraft took him to Christchurch, New Zealand, for treatment.

US efforts to drop medical supplies to the other sick man, who had suffered a heart attack, failed and a Soviet aircraft will take him to New Delhi.

DOD officials said that this was the first time in eight years that a catastrophe of these proportions had struck an Indian expedition. But the depleted team is expected to continue the planned research activities.

K.S. Jayaraman

CREATIONISM

Approval for degrees turned down

San Francisco

ALTHOUGH a final decision will not be made for several weeks, the state of California is expected to deny approval for the Institute of Creation Research Graduate School (ICRGS), located near San Diego, to issue masters' degrees in science. The recommendation comes after more than two years of investigation into ICRGS, and had been expected by both the state department of education and the school, which offers masters' degrees only and holds courses solely during the summer.

Under California law, non-accredited institutions must receive state approval to issue academic degrees by showing they offer coursework and facilities comparable to accredited institutions, according to William Ruckeyser, special assistant to the state superintendent of public instruction. ICRGS, which has ten full-time faculty members and taught about 20 students last summer, has been receiving permission to issue masters' degrees in astrogeophysics, biology, geology and science education.

Academic investigative teams visited the school in 1988 and again last summer, ultimately recommending against re-approval. Their latest findings will be brought to the state Council for Private Post-Secondary Educational Institutions, which then reports to California's superintendent of public instruction, Bill Honig, who makes the final decision. But Honig has already indicated he will follow the recommendation, said Ruckeyser.

The decision has been praised as a victory for science over creationism. "The committee made the right decision for the



right reason", said Eugenie Scott, executive director of the Berkeley-based National Center for Science Education, who asserted that coursework at the institute was far below today's scientific standards. "The ICR was rejected because they do a terrible job of training students. Any institution in California with as poor a program as the ICR would also be rejected." Anticipating ultimate rejection, John Morris ICRGS vice-president, challenged the objectivity of the latest investigative committee and declared that "they came with an agenda to shut us down". Listing courses that include earth structure, tectonics, and sedimentary geology, Morris said, "We are a Christian school, but we teach science". He indicated that the school will seek an administrative appeal to the expected decision.

Robert Buder

Information service goes

Sydney

MONEY earmarked for the Australian Science and Technology Information Service (ASTIS), a science resource for the general public and the media, has been cut from the government science budget, allegedly to pay for ballooning expenses in the administration of the Australia prize. The prize is a new A\$250,000 international award to be given in the biological sciences related to agriculture and the environment, and its first recipient will be announced on 9 April.

The federal government was advised by the Australian Academy of Science (AAS) as early as 1984 that the administrative costs of the Australia Prize would run to A\$200,000. These costs are now admitted to be A\$160,000 for the first year and A\$100,000 for each subsequent year. Nevertheless, the May 1989 budget mentioned no administration costs, only the cash value of the prize, and the scientific community had become increasingly apprehensive that the large sums needed

France and Israel stay close

Jerusalem

DEFYING a recent call by the European Parliament to boycott scientific cooperation with Israel, France last week signed a \$2-million binational agreement for joint research over the next two years. According to the terms of the agreement, each country will spend \$500,000 for joint research in supercomputer hardware and software and \$500,000 on biotechnology.

The European Parliament last month passed a resolution asking the European Commission (EC) to freeze funds earmarked for scientific cooperation with Israel as a protest against the continued closure of Palestinian universities in the occupied territories.

The Israeli Ministry of Science and Technology responded by saying that if Israeli and European scientists were prevented from cooperating on research projects, the reverberations would go well beyond Israel's borders. Arye Shumer, the ministry's director-general, said that such a move could be "lethal", and advised the European Parliament to consider "who would really be hurt" by a boycott. He mentioned the extensive and close ties between scientists here and their counterparts in Europe, and added that the EC is one of the most important sources of funds for Israeli universities.

The agreement with France brought a sigh of relief in Israel. Shumer said it proved that scientific ties with the EC were unbroken.

Lisa Perlman

would be lost from the science budget.

A spokesperson for the Department of Science said that the funding for the Australia Prize had been "allocated from the start" and that if the prize were cancelled "the money wouldn't have gone back to the science budget". But according to the opposition spokesman for science, Peter McGauran, the axing of the science information service ASTIS, which was to have been supported to the extent of A\$400,000 a year for three years, is a consequence of the unaccounted costs of the Australia Prize.

Professor Bob Crompton, the chairman of the AAS working party charged with developing the ASTIS proposal, wrote to the *Canberra Times* that "the impact of an information office servicing the media and therefore the public for 365 days a year would be greater than that of a prize, no matter how prestigious, that catches the public attention fleetingly once a year". The Minister for Science, Barry Jones, has been increasingly critical of ASTIS, saying that similar US and UK media resources deal with only 4,500 and 1,600 calls a year respectively, and that ASTIS, because of Australia's smaller population, would receive no more than 500 calls a year.

In a letter to the AAS president David Curtis, Jones argued that that "when

UNIVERSITY OF CALIFORNIA

Challenge to expansion from private colleges

San Francisco

PLANS to create three new University of California (UC) campuses by the year 2000 have met with a carefully crafted challenge from the state's private institutions, which argue that less-costly financial incentives might enable under-enrolled independent colleges and universities to absorb the new students expected by the UC system.

Taking pains to emphasize its wish to work with UC, the Association of Independent California Colleges and Universities (AICCU) is lobbying hard to bolster the Cal Grant programme that provides financial aid to California high-school graduates and could help them offset the cost differences between public and private institutions.

The association is well aware that the state department of finance has projected that UC must make room for 67,500 new students by the year 2005. "Everybody agrees that UC has to build at least one new campus to accommodate those students", said Hans Giesecke, director of marketing and research for AICCU, which represents some 90 accredited four-year institutions. "The question is do they need two or three new campuses?"

Association leaders take their case before a state assembly committee on 13 Febru-

science-related stories blow up, for example Voyager 2, cold fusion, gene shears and the San Francisco earthquake, a mass of readily available information is generated very quickly. . . . The experienced ones won't need [ASTIS] and the novices are unlikely to try." But Alan McGowan, who is president of the US media service SIPI (Scientists' Institute for Public Information), has written to the executive officer of ASTIS to say that Jones is mistaken: "Every independent observer of the media resource service here has commented on its success and its growing importance in explaining science to the public". In fact, ASTIS originally arose out of comments made in 1984 by Jones, who called on scientists to communicate with the public and with the press. In the past three years an ambitious feasibility study has been completed with the financial support of every Australian university, the Commonwealth Scientific and Industrial Research Organization (CSIRO), the Department of Industry, Technology and Commerce, of which the Ministry of Science is a part, and with additional corporate sponsorship of A\$330,000 over three years.

Despite the government's abandonment of the proposed service, Curtis says AAS "has not deserted the idea of ASTIS. . . . The US service succeeds without relying on the government, and so can we."

Tania Ewing

ary, on the same day that a UC board of regents will meet to recommend where in the state the first new campus should be. AICCU studies note that while some of its members are at full capacity, many are not. By changing the formula by which the Cal Grant awards are made, the association hopes to reverse that trend.

The grants are awarded in different amounts based on need and can be used for either public or private institutions. The current maximum award of \$5,250 a year is enough to pay UC costs but pays less than half the average AICCU member school's tuition and fees, said Giesecke. Association figures indicate that a boost to Cal Grant could enable its members to take up to 31,000 new students by 2005. Not all would come from the same pool as the projected UC students, but the group believes many would — supporting its call for a closer look at the UC expansion.

UC officials say the private school situation is a side issue. "We've taken their projections into account in our projections", said a system spokesman. Giesecke countered, however, that the UC plans do not assume a more favourable Cal Grant situation and that the matter is still unresolved.

Robert Buder

Australia turns to the East

Sydney

AUSTRALIA's plans for a A\$13,000 million futuristic city populated by high-technology workers from Japan and other Asian countries took a step closer to reality last week with the release of a consultant's report.

The study of the 'Multi Function Polis' by Andersen Consulting and Kinhill Engineers cost A\$4.5 million and describes the new city as a "major urban centre of the future with a diverse economic base of knowledge-intensive industries built around a core of education-based activities". The city is essentially an attempt by Australia to internationalize its economy by selling its service industries.

On offer are Australia's strengths in seven areas: education; health; transport; leisure, entertainment and media; information and telecommunications; environment and agriculture; and construction and design. The prize is the skilled workers and professional staff who are at present emigrating to Canada and the United States from Asia.

"Good service industries such as education and health together with a

pleasant lifestyle", could turn Australia into a beacon for Asian emigrants, according to Terry Hilsberg, previously first secretary of the Department of Technology, Industry and Commerce (DITAC), and a member of the committee that thought up the technopolis.

The consultant's report favours a single site, to be announced in June, for a city similar to Tsukuba science city in Japan. While a single site, with its central physical infrastructure, is considered more enticing for the overseas investors that will be vital to the project, it sets problems for Australia's dispersed resources — strength in biotechnology is concentrated in Melbourne, in information technology in Sydney and in materials science in West Australia.

A privately funded international university with a population of up to 200,000 from Australia and overseas will provide the core of the city. Infrastructure costs are expected to be provided for by the rise in land values once the site has been announced, according to Hilsberg.

Professor Gavin McCormack of the Centre for Asian Studies at Adelaide University says that Japanese companies will

be asked to invest in certain sectors only after the site has been chosen. According to a spokesperson for DITAC, about 80 Japanese and 80 Australian companies have expressed interest and have invested money in the feasibility study.

The city is thought likely to generate up to 30,000 highly skilled jobs. The idea of a "multinational technology park bringing together high technology and other brain-based industries" originally arose four years ago out of a proposal by Japan's Ministry of International Trade and Industry (MITI) for a leisure park in Australia for overworked employees.

The Japanese have experience with large-scale urban planning. In the 1950s, several new towns were created to accommodate heavy industry; in the 1970s the emphasis shifted to planning for knowledge-based industry.

According to Hilsberg, however, Australians might be uneasy with following the Japanese way. "There are two problems — Australians see their future as lying with United States and Europe and still tied up with primary industry, which is a very fragile base for our economy — our future in fact lies with offering service industries such as health and education, which could be big export earners, to Asia."

Tania Ewing

ASTRONOMY

New northern sky atlas

Munich

THE European Southern Observatory (ESO) and Palomar Observatory, California, announced on 26 January that they would collaborate in producing the first new astronomical atlas of the northern hemisphere sky in over three decades. The observations, a series of 2,682 photographic plates that cover the whole northern sky, will be made with Palomar's refurbished 40-inch Oschin Telescope.

Later this year, ESO will begin making high-quality copies of plates. The resulting sky atlas, which will show stars up to seven times fainter than those in the current Palomar sky survey, will become a standard reference for astronomers worldwide.

A spokesman for Palomar said it would be less expensive for ESO to produce the atlas than it would be for Palomar itself, a result of ESO's long experience in the reproduction techniques required. ESO astronomer Richard West, who will supervise the project, said it was "an honour" that ESO had been asked to produce the atlas, and that as a bonus it would be able to provide cheaper copies of the atlas to institutes in the ESO member states.

The atlas will take 10 years to produce. Glass copies, of which fewer than 10 will be produced, will cost DM460,000 (about \$270,000). Film copies will cost a mere DM60,000.

Steven Dickman

RADON LEVELS

More UK homes naturally at risk

London

THE number of homes defined as having 'potentially hazardous' levels of naturally occurring radiation may rise from 25,000 to 75,000, following the National Radiological Protection Board (NRPB)'s decision to halve its 'action level' for exposure to radon gas.

Radon, seeping up into buildings from uranium-bearing rocks, is the main source of human exposure to ionizing radiation, and is thought to cause some 2,500 deaths from lung cancer in the United Kingdom each year — the second most important cause of this disease after cigarette smoking.

NRPB first issued advice on radon exposure in January 1987. This was taken up by the government, and set the action level at 400 becquerels (Bq) per cubic metre. For average radiation exposure above this level, householders were advised to make simple alterations — blocking up cracks in floors through which radon could seep, and improving under-floor ventilation — in many cases to be paid for by the government. A lower level, 100 Bq m⁻³, was suggested for newly built houses.

The new recommendations, released on 12 January and accepted by the government, suggest a single level of 200 Bq m⁻³ for all homes, and are based on surveys of lung cancer rates in uranium miners. These suggest that the risk posed by radon may be



two to three times greater than thought previously.

The NRPB suggests that monitoring of radiation levels should be concentrated on 'affected areas', where it is likely that more than one per cent of homes fall above the new action level. Most of these will be in the southwest of England, but the new action level means that parts of Derbyshire and Northamptonshire, in the English Midlands, may also be included.

The NRPB's advice will still not bring the United Kingdom in line with the United States, which adopted a level of 150 Bq m⁻³ in 1986. But Michael O'Riordan, head of NRPB's radon programme, said that the new action level set a proper balance between the risks involved and the feasibility of solving the problem. "Adopting a lower level would . . . mean that attention might not be focused on those parts of the country with the worst problems."

Peter Aldhous

The career of F.W. Twort

SIR—By using the title 'What happened?' to R. A. Slepecky's letter¹, you seem to be inviting further comment on the career of F. W. Twort. His work after the discovery of bacteriophage in 1915², his ideas about biological replication and the relationship of organisms to one another, and his frequent troubles with sources of finance, are described in papers by him in 1936³ and 1949⁴, and in the obituary by Sir Paul Fildes in 1951⁵.

His discovery of bacteriophage was a logical consequence of his earlier work on John's bacillus, which would not grow in culture media although it closely resembled the tubercle bacillus which grew readily. He reasoned that the latter was able to make an essential growth factor and that this would enable John's bacillus to grow. By adding killed tubercle cultures, or extracts from them, to the medium, he got John's bacillus to grow and made a practically useful diagnostic vaccine. Extending this principle to viruses, he cultivated the various bacteria present in commercially available glycerinated vaccinia lymph and tried to get the vaccinia virus to grow on these bacteria. The logic was imperfect because there was no reason to think that these bacteria had any evolutionary or developmental connection with vaccinia and had not merely strayed into the lymph during commercial production, but the patches of transmissible bacterial degeneration were unmistakable.

His ideas about evolution, competition and interdependence made him assume that viruses which did not overtly infect hosts, and previruses, were omnipresent. The problem was to get them to multiply to an extent that would make that presence obvious. This is the main theme of his 1936 paper. In an attempt to recreate conditions analogous to those he thought typical of probiotic Earth, he reflected light from many different minerals on to many potential substrates. It is interesting to note the important place of clays (to which interest is now returning) from various sources in these experiments. In his 1949 paper, he wrote "... by 1939 I knew that the technique which was being evolved was correct. Striking results of a positive nature were obtained, but, unfortunately, in 1944 the laboratories of the Institution were destroyed by a bomb, and the University deprived me of my post and all facilities for completing the research." It is a pity he did not say what sort of results he was getting. Nevertheless, as I have argued elsewhere⁶, it might be worthwhile setting up similar mixtures and examining them every few years for signs of change.

In his 1936 paper, he stressed that he was trying to identify novel or latent forms

of life rather than its creation. He became more ambitious later. Soon after Twort's death, someone (presumably Fildes) lent me a long typescript which discussed biopoeisis. According to my memory, it made no points with which I was not already familiar. I may be wrong: Twort obviously thought he was being original. In those days, photocopiers were not part of normal laboratory furniture; I returned the typescript uncopied. If a copy still exists somewhere, it would be interesting to see how well, after 40 years, the ideas in it agree with those that have now become conventional. Twort's intuition was productive about John's bacillus and about the potentialities of vaccine lymph. Perhaps what Fildes called the "admirable confusion" of his work on clay and other minerals was not as daft as most of his contemporaries thought.

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SIR—I can tell Ralph A. Slepecky that I have prepared a biography of my father, Frederick William Twort (1877–1950) and am currently negotiating for publication. My father's life was never dull but full of troubles, many of them relating to lack of proper support for his imaginative researches in microbiology. He was a man of many parts who suffered the greatest frustration. But he did none of the things suggested in Slepecky's letter. His constant preoccupation with scientific thoughts would have made him a very bad London taxi driver, but he would have enjoyed the challenge of designing a better vehicle.

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Thinking machines

SIR—Richard Gregory twits physiologists for not sharing his mechanistic views (Nature **342**, 471; 1989). They must answer for themselves, but they are probably less frightened of being branded alchemists than of being considered neo-vitalists. In August (Nature **340**, 517; 1989), you printed a fine colour photograph of the Science Museum's recent construction of a sub-unit of Babbage's Second Difference Engine. What a pity

you did not reprint it to illustrate Gregory's article. It might have helped your readers to decide whether *any* machine composed of such elements (or of electronic ones), however complex its specification, can properly justify Gregory's assertions that Babbage "showed that a machine could make *its own* decisions during calculations" (emphasis added) — and that today's computers can "see".

More helpfully, Gregory does state elsewhere in the article (page 472) that Newton "found the mechanistic account of nature in terms of forces and motions of bodies unsatisfactory as a complete account". Perhaps physiologists should enquire whether physicists agree with Newton or with Gregory.

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SIR—I should like to add to Richard Gregory's fascinating and interesting article on Newton and alchemy (1989). It is true that Newton was the posthumous offspring of a farmer, but Newton's mother, Hannah, was fairly rich, because of the fortune she inherited from her second husband, the Reverend Barnabas Smith, who died in 1653. She married him in 1645, when Newton was barely three years old, and for about eight years Newton lived with his grandparents and hardly saw his mother. This forced separation and his total rejection by his stepfather embittered Newton all his life, and he never got over the loss of his mother's love. While at Cambridge, Newton had to wait on tables in the dining room, although Hannah could easily have paid all his expenses. His unhappy childhood is probably responsible for Newton being a bachelor and dying a virgin.

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Mirabile dictu

SIR—I was amused to read in a recent leading article your statement that a single well-authenticated case of water running uphill would be a scientific observation well worthy of publication.

I occasionally go for my holidays to a valley in the West of Ireland where this phenomenon is a common sight. Winds blowing in off the Atlantic Ocean are funnelled by the valley walls and intensified to such an extent that it is not unusual to note a mountain stream reversing direction, or even a waterfall turning into a fountain.

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An alternative to academic boycott

Solomon R. Benatar

How can the international academic community contribute to the demise of apartheid? Academic boycott is a political strategy of doubtful ethical basis, and should be replaced with a policy of selective support.

THE history of South Africa during the twentieth century can be described as a combination of industrial and economic success intimately coupled to racially discriminatory political and social processes that have denied most of its population access to recognized human rights¹. South Africa's economic and other successes — by comparison with the rest of Africa — have been totally eclipsed by its tragic political failures, by its refusal (as a nation supposedly committed to the Western tradition) to identify with the Universal Declaration of Human Rights, and by legislation which has relentlessly silenced much opposition, eroded the rule of law, entrenched the ruling party, muzzled the press and degraded human dignity. These deficiencies constitute the basis for South Africa being progressively ostracized by many Western countries.

Academic boycott, which has operated informally for many decades, has recently become consolidated into a more formal component of a strategy to pressurize the South African government to reverse its repressive and discriminatory policies and to develop a non-racial democratic society. Academic boycott, examples of which in the medical and scientific fields include banning participation in congresses, refusals to lecture in South Africa and censoring of publications, has stimulated a plethora of conflicting views². Last week's announcement by President F. W. de Klerk — that political reform is to become a reality — will fuel rather than dampen the arguments.

It is important to understand and to empathize with the frustration, anger, despair and moral outrage which have led to advocacy for academic boycott as part of a political strategy to achieve desperately needed changes in South Africa. It is also important to strive for a more just society in South Africa. Nevertheless, it must be acknowledged that the forces motivating and driving political strategy are not necessarily the same as those that motivate scientists, physicians and educators in their professional roles.

Although moral outrage against the racist policies of South Africa, with all the devastating effects these have had, is clearly justified, the consequence that academic boycott is a morally imperative response requires more than mere assertion. The potential of such a boycott to affect adversely higher education, medical education, scientific endeavour, health

care and the international collaborative spirit of higher education, all of which are necessary both now and in a post-apartheid South Africa, cannot be ignored. Many who reject and oppose apartheid, who acknowledge their own unwitting enmeshment in the process, who agree that it is beyond dispute that the hegemony of a white power oligarchy

academic boycott is a moral imperative, the authors of this document also express growing concern for the adverse effects of such sanctions. They offer guidelines as a basis for "selective support" of academic exchanges. If selective support means freedom of choice in targeting one's support of colleagues in South Africa, and is not a euphemism for selective boycott

³ (requiring coercion for its implementation), this is certainly a more justifiable alternative to total boycott or uncritical support. (A subsequent statement from the African National Congress re-affirmed advocacy for selective academic boycott.)

Can academic boycott be justified within the traditional framework of professional moral codes? The World Medical Association (WMA), which has been active in the formulation of

Cape Town University students clash with campus security as they try to disrupt a lecture from a visiting scholar in 1986.

cannot continue in South Africa and that all political skills need to be brought to bear on achieving peaceful changes, consider that there are more constructive approaches than academic boycott.

Because of the moral outrage against apartheid policies, discussion tends to be emotive, inconsistent and characterized by attempts to conflate political strategy with moral justification. Strategically justifiable political actions may indeed have a moral component, but I question the assertion that academic boycott is necessarily part of the moral imperative. I suggest that pressures generated through imaginative programmes of targeted professional support have greater moral consistency, justification and potential than academic boycott as a means of facilitating change in South Africa.

Selective support

The background and rationale for academic boycott is stated, and the arguments used by opponents and proponents outlined, in a recent publication³ from the National Medical and Dental Association (a progressive medical organization formed in 1982 as a result of discontent with the Medical Association of South Africa's actions following the death of Steven Biko). Although asserting that

such codes, has rejected academic sanctions on the grounds that they conflict with the main objectives of the association — "to achieve the highest international standards in medical education, medical science, medical art and medical ethics" and such restrictions will "adversely affect health care, particularly of the disadvantaged" (ref. 4). The WMA's codes on various issues are widely accepted. Those who are willing to reject its view on academic sanctions must realize that this could erode the force of other WMA declarations, such as the role of doctors in war or caring for prisoners.

Although professional codes stress the need for collegial cooperation and support, recognition that there may be circumstances in which the imposition of sanctions may have to be considered has been addressed by the Council for Science and Society in collaboration with the British Institute of Human Rights⁵. "If any sanction is to be effective to achieve its special object, and is not to devalue the effect of other sanctions to be applied on other occasions, it must be appropriate (directed to the removal or mitigation of the particular evil which it is sought to combat), efficient (calculated to have the desired effect) and proportionate (use no more external force than is needed to

IMAGE
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achieve its effect).” In addition to conforming to the principles of appropriateness, efficiency and proportionality, sanctions “must not be imposed in such a fashion as to reduce the imposer of the sanction to the moral level of the wrongdoer whose conduct is under criticism. Whoever wishes to take steps to protect scholarly freedom must not himself imperil that freedom by the steps which he takes.”

Given the consistent opposition of many South African universities, professional bodies and individuals to apartheid and racial discrimination, it seems that the accusation that academics in South Africa are guilty of “colluding” with initiating, maintaining or supporting government policies that lead to violation of human rights is an overstatement. That many academics have been complacent about the socially destructive society in which they live as privileged members is beyond dispute. But it should also be acknowledged that individuals, professional bodies and academic institutions in ‘free’ countries (many of whom are also complacent about social injustices in their own environments) have either remained aloof or have devoted much more effort to a condemnatory stand than to encouraging or assisting their South African colleagues to oppose more actively violations of human rights and thus to contribute more effectively to change. Under these circumstances, it is not readily apparent that academic sanctions can be considered as appropriate or proportionate.

Academic freedom, even in its imperfect form in South Africa, needs to be protected if universities are to survive into a post-apartheid society. This view will be disputed by those who are scornful of the ‘liberal’ position or who consider even the open universities in South Africa to represent white, racist institutions that by their very nature perpetuate injustices in our society.

Concern for the role of politics in science has been expressed by several prestigious organizations⁵⁻⁷. The committee on Human Rights of the US National Academy of Sciences has acknowledged the difficulty in grappling with the continuum of repression and in determining just what is science and what is political. But it affirms that its position continues to be to hold to the universality principle, not to restrict the channels of scientific communication in the name of trying to maintain and extend them⁷.

Ethics

An alternative means of exploring the moral validity of academic boycott is to question whether it can be justified within an ethical theory. One general tradition focuses on ethical principles, which subtend and justify right actions independent of their consequences, and on the moral

integrity of individuals faced with making moral decisions. Specific actions are considered ‘right’ if they conform to an overriding moral duty (principle) and ‘wrong’ if they violate such duties.

A chief deficiency of such theories is their failure to provide guidance on how one ought to act when two or more moral principles are in conflict. In relation to academic boycott, for example, it could be argued that to distance oneself from academics in a repressive country is a morally principled means of expressing moral outrage, of separating oneself from evil and thus preserving moral integrity. But it could also be argued that actively to support education and academics who oppose the repressive regime is equally principled. How does one choose between these two courses of action? To choose the former may provide quick emotional satisfaction, but this would be an arbitrary rather than a reasoned choice.

These difficulties lead to the consideration of an alternative group of ethical theories which argue for the selection of a course of action which will lead to the greatest net balance of ‘good’ over ‘bad’ outcomes. In this context it is the ends which justify the means. The central criticisms of this group of theories are that weighing and balancing principles tends to sacrifice the individual for the common good, and that it is not possible to know without empirical evidence whether good consequences will be maximized in the short or long term. Crucial to this utilitarian notion is the time within which the anticipated change will occur. Despite recent encouraging developments, the time frame in South Africa is likely to be sufficiently long to confirm the already obvious shortcomings and adverse effects of the cry of “liberation before education” which seriously undermine the hoped for effectiveness of the academic boycott^{8,9}.

Yet another approach is one which enhances individual discretion by allowing for greater flexibility in reaching moral decisions. But, for example, selectively boycotting those academics who are not opposing the repressive regime is an unworkable approach because of the difficulty in determining the criteria for identifying those who fall into this group and problems of implementation. The coercion involved is also inimical to academic freedom.

The sincerity of many who advocate or impose sanctions should not be doubted. Unfortunately, an unintended by-product of academic boycott is the creation of opportunities for self-serving activities by individuals which have the potential to brutalize science and to lead to great damage, in particular over a prolonged period of time. The sincerity of many who oppose academic boycott should also not be doubted, but freedom must be used responsibly and in ways which do not

curtail the freedom of others — consistent with the concept of freedom built over centuries into the ‘social contract’ within the Western tradition.

Alternatives

Living and working in South Africa presents the onerous task of challenging apartheid, striving towards abolishing racial discrimination and towards achieving internationally recognized human rights. Consistency and clarity in articulating and trying to uphold these ideals is demanding and poses risks which at a minimum include unpopularity with the government of the day. The strength and vigour of endeavours to uphold ideals with constancy (wherever this is needed) is dependent on international cooperation and support not only for those already committed to this ideal but also for those who could be motivated to do so^{3,5,7,8}.

Apartheid, racialism and all forms of discrimination which degrade human dignity must be rejected. Great effort has been expended and much more is needed to create a democratic, just society in South Africa. The strength of professionals’ special contributions to this society should not be diluted by conflating political imperatives with moral imperatives. The assertion that academic boycott is ‘moral’ requires that its ethical basis should be clearly justified, and it is those who wish to impose restrictions on freedom who must do this. Imposing academic sanctions without ethical justification can reduce its protagonists to the moral level of the regime of which they are critical and can serve further to isolate and alienate those who are endeavouring to contribute to change in South Africa from within.

I propose an alternative — pressure for change through freely offered support on a selectively targeted basis (rather than resorting to the coercion associated with selective boycott) — as a more ethically justifiable approach. That this will require greater involvement, more commitment and greater effort is undisputed, but these are indeed the requirements for moral action, as distinct from moral posturing. □

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Tectoclimatology comes of age

Computer modellers have successfully simulated the effects of major mountain-building in the past 10 or 40 million years on the climate we now experience.

THAT we should all now be climatologists, if only from our armchairs, is not surprising, with the prospect that an excess of greenhouse heating may provoke climatic change. But there are still virtues in the long view, especially in efforts to understand the causes of the marked climatic changes evident in the fossil and geological records, the Pleistocene glaciations in particular. For who can tell that these are irrelevant to more immediate worries?

That is why a group of three articles in a recent issue of the *Journal of Geophysical Research* (the purple version, discreetly labelled "Atmospheres" on the spine) deserves more attention than it is likely ordinarily to command. The question is whether changes in the topography of the surface of the Earth in the past 40 million years have influenced the climate and, if so, how. That the Late Cenozoic has been marked by profound changes of topography, with the dramatic uplifting of the western third of North America and of the Tibetan Plateau together with the Himalayas, is not disputed among geologists. The surprise is that the climatic consequences can be traced in rich detail by computer models.

That topography (more strictly, orography) should have a regional influence is credible enough. There is a strong thread of surmise going back to the nineteenth century that the summer monsoon in India is partly driven by the rising column of heated air above the Tibetan Plateau. (So much is now confirmed.) Whether the climatic consequences of orogenesis can be global in character as well as extent remains an open question, although the past 40 million years must be as good a candidate as any previous period.

The distinctiveness of this period is put well by W.F. Ruddiman and M.E. Raymo from the Lamont-Doherty Observatory at Columbia University and W.L. Prell from Brown University (94, 18, 379-391; 1989). The Tibetan Plateau has been uplifted by up to 4 km in the past 40 million years and by at least 2 km in the past 10 million years. Similarly, two-thirds of the uplift (and southwest tilt) of the Sierra Nevada has happened in the past 10 million years. But much the same applies to the other mountainous provinces of the American West. The Colorado River, for example, has downcut the Grand Canyon by 1 km in the past 10 million years, a third of that in the past 1.5 million years. Many of these

tectonic processes, the uplifting of Tibet for example, are still under way, as (in the Southern Hemisphere) are the uplift of the Bolivian Andes and, probably, the New Zealand Alps.

How to model the consequences of these changes? Ruddiman and Prell, with J. E. Kutzbach and P.J. Guetter from the University of Wisconsin at Madison, describe their use of the Community Climate Model (CCM) at the National Centre for Atmospheric Research (NCAR) to tell what happens (94, 18, 393-407; 1989). The first step is to model the orography then and now, which is a matter of parcelling out estimated Cenozoic uplift into the geographical blocks (4.4° latitude by 7.5° longitude) used by the CCM. There is a "no mountain" case in which present-day mountains are sliced off at 400 metres, a "half-mountain" case in which the added uplift is half of that known to have occurred and a "mountain" case representing the present.

Inevitably, the computer simulations are artificial—true experiments. Only the Northern Hemisphere is modelled. Insolation is fixed for either 16 January or 16 July, robbing the system of the effects of seasonal oscillation. Sea-surface temperatures are held fixed, and no account is taken of changes of the proportion of the oceans covered by ice or, for that matter, changes in the extent of ice-caps (which means that glaciations are excluded). The trick is to run the model for the equivalent of 900 days, exclude an initial transient period and to select 90-day intervals from the resulting record of changing weather so that, when internally averaged, they serve as independent estimates of the climate. The end result is a synoptic representation of average sea-level pressure, wind speed and the like for January and July.

Ruddiman and Kutzbach (94, 18, 409-427; 1989) take on the task of saying what this means, but some things are clear even from an armchair. First, the pattern of the average wind-velocity is uniform and symmetrical when there are no mountains, but much distorted when there are. With no mountains, planetary waves tend to be anchored at the boundaries between continents and the oceans, but mountains make the high plateaus dominant.

The details will provide endless fascination, not only for climatologists but, for example, for palaeobotanists. Here are some of the inferences to which Ruddi-

man and Kutzbach commit themselves. The dry-summer mediterranean climate of the western seaboard of North America in mid-latitudes is, for example, caused by the conversion of the westerlies expected with no mountains into northerly winds flowing south from British Columbia to the Mexican border, associated with a deepened pressure low over the Colorado Plateau.

The other side of the Rockies, the other side of that coin is that both seasons are wetter than previously, the winters because the jet stream is shifted south and the summers because of the monsoon flows engendered by the Colorado low. East of the Rockies, the winters are also now colder than 10 or 40 million years ago, chiefly because previously west winds are now northwesterly. Even Florida has not escaped the consequences of the great Cenozoic orogeny; both winter and summer are wetter now.

One sign that the consequences of uplift can be global is the inference that northern European winters are now colder and Mediterranean summers are drier than they used to be. The first effect is a result of the westward displacement of the statistical Icelandic low, the second a distant consequence of the huge cyclonic flow around the Tibetan Plateau and, simultaneously, of a high above the subtropical Atlantic. For what it is worth, uplift has made Central Asia colder and more arid than it used to be.

Part of the interest of the tale is that it can be compared with what is known from the palaeobotanic record. There were, for example, magnolias (which need water in the summer) growing wild in California late in the Pliocene. The disappearance of broad-leaved evergreen forests such as still flourish in temperate southern China is attributed to the severe winters caused by the seasonal influx of polar air.

So what is to be made of the exercise as a whole? Its most striking feature is that such a wealth of detail can be wrung by skilled interpretation from a computer model whose elements are as rudimentary and whose assumptions are as crude. It is presumably now a challenge to see whether the model can be given the extra components of realism necessary to let icefields form, which will require an ocean that plays a part in weather. Meanwhile, there could hardly be a more vivid proof that the world's climate hangs together as a whole.

John Maddox

Scavenging for receptors

Michael S. Brown and Joseph L. Goldstein

OUR current understanding of atherosclerosis is rather like our understanding of cancer 25 years ago, before the discovery of oncogenes provided a unifying hypothesis for various causes of the disease. Several causes of atherosclerosis have been identified, including cholesterol-carrying lipoproteins, cigarette smoke, hypertension, age and hereditary factors, but no unifying theory has emerged. Now, on pages 531 and 570 of this issue^{1,2}, Krieger and co-workers take us closer to such a theory by reporting the cloning of the complementary DNAs for two scavenger-cell receptors from macrophages.

Scavenger-cell receptors were discovered in 1979, during attempts to learn how cholesterol from low-density lipoprotein (LDL) accumulates in macrophages in atherosclerotic plaques of patients with familial hypercholesterolaemia (FH) who lack LDL receptors³. Such cholesterol-loaded macrophages, termed foam cells, are conspicuous in arterial plaques from FH subjects and other individuals with high levels of LDL in the plasma. Isolated macrophages from normal and FH subjects had few LDL receptors and failed to take up native LDL; but they avidly took up LDL, the lysine residues of which had been modified *in vitro* by chemical acetylation. This converted them into foam cells^{3,4}.

Cell-surface receptor

The key to macrophage foam-cell formation turned out to be a cell-surface receptor; this receptor recognizes the increased negative charge that is unmasked on the LDL protein when the positive charges on lysines are abolished by acetylation³. Uptake of acetyl-LDL is inhibited competitively by some, but not all polyanions, from which it would seem that the receptor recognizes conformation as well as charge. Particularly striking is its ability to distinguish single-stranded polynucleotides: polyinosinic and polyguanylic acids bind to it, but polyadenylic and polycytidylic acids do not⁴. Because of its wide specificity, the acetyl-LDL receptor soon became known as the scavenger receptor.

The search for a physiological ligand for the scavenger receptor bore fruit when Steinberg and co-workers discovered that oxidized LDL competes for the binding of acetyl-LDL and delivers sufficient cholesterol to produce a foam cell (reviewed in ref. 5). The complex LDL particle comprises a cholesteryl ester core surrounded by a hydrophilic coat containing hundreds of phospholipid molecules and one protein (apolipoprotein B-100). The unsaturated fatty acids of the phospholipids are

highly susceptible to chemical oxidation, which is prevented in plasma by antioxidants. It is thought that these antioxidants become depleted when LDL is trapped for a prolonged period in artery walls⁵⁻⁷. The LDL is then left to the mercy of oxygen radicals that convert the unsaturated fatty acids into reactive aldehydes and oxides that attach to lysines of apoB-100, mimicking acetylation^{5,6}. Such oxidized LDL is toxic to a variety of cells *in vitro*⁷, and its importance *in vivo* has been dramatically underscored by three lines of evidence — first, antibodies against aldehyde-conjugated lysines stain atherosclerotic plaques^{8,9}; second, LDL from atherosclerotic plaques has an increased negative charge, binds to scavenger-cell receptors and produces foam cells *in vitro*^{9,10}; and third, probucol, an antioxidant, partially prevents atherosclerosis in Watanabe-heritable hyperlipidaemic rabbits, an animal counterpart of FH^{11,12}.

From cross-competition experiments, it seems that macrophages express several scavenger receptors^{13,14}. One class prefers acetyl-LDL, another prefers oxidized LDL and a third binds both. A possible explanation for this diversity is to be found in the observations reported in this issue^{1,2}. Following up on the original chemical identification by Via *et al.*¹⁵, Kodama *et al.*¹ and Rohrer *et al.*² isolated the scavenger receptor from bovine lung, determined a partial amino-acid sequence, and cloned two closely related cDNAs from a lung library.

The receptor appears to be a trimer^{1,2,16}. Each highly glycosylated monomer of 453 amino acids (type I receptor) or 349 amino acids (type II receptor) possesses one membrane-spanning region and has its N-terminal 50 amino acids in the cytoplasm and its C terminus outside. The external region of both receptors contains an unbroken stretch of 24 Gly-X-Y triplets, where many Ys and some Xs are prolines. This structure is reminiscent of fibrous collagen, the monomers of which contain 338 Gly-X-Y repeats that wrap around each other to form a triple helix. Short stretches of collagen-like repeats are found in a few other extracellular proteins, including acetylcholinesterase and complement component C1q, but these are the first cell-surface proteins that have been found to be so adorned. As well as including the collagen-like repeats, it is postulated that the two scavenger-cell receptors contain 23 heptad repeats, raising the possibility of the existence of an additional region of α -helical coiled-coil interaction. The only structural difference in the two receptors is that the type I receptor contains an extra cysteine-rich

sequence of 110 amino acids at the C terminus. Whether the two types of scavenger receptor form homo- or heterotrimers is unknown.

When transfected into COS cells, each cloned receptor bound acetyl-LDL with similar affinity and showed the expected specificity of polyanion binding (that is, competition from polyinosinic acid, but not polycytidylic acid). A crucial question, not yet answered, is whether one or both receptors binds oxidized LDL. Do these two receptors account for all of the scavenger activity of macrophages or should the scavenger hunt be continued?

Collagen

Other questions present themselves. Is the resemblance to collagen merely another instance of nature re-using old exons for new purposes, as happens so often with other cell-surface proteins? Or is there a functional resemblance between the scavenger-cell receptor and collagen? The second possibility finds support in the formation of trimers and the wide binding specificity. As the authors remind us^{1,2}, collagen binds many molecules, including oxidized LDL¹⁷ which is deposited in collagen-rich tissues such as tendons and atherosclerotic plaques. The scavenger receptor might compete with collagen for binding oxidized LDL and perhaps other toxic molecules. Macrophages might glide along collagen fibrils, using their scavenger-cell receptors as vacuum cleaners to suck up oxidized lipoproteins and other polyanions that are stuck to collagen.

Irrespective of any functional resemblance to collagen, the scavenger receptor may be an important defence mechanism

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that enables scavenger cells to ingest and degrade oxidized and chemically altered proteins. This would explain its occurrence in wandering macrophages (monocytes), which invade poorly perfused tissues, such as tendons and atherosclerotic plaques, that may become depleted of antioxidants. It might also explain the receptor's unusual abundance in the endothelium of hepatic sinusoids¹⁸, where it might act as a filter to remove oxidized proteins from the bloodstream.

Although the scavenger receptor may play a protective role in most tissues, in the atherosclerotic plaque it may cause damage^{3,5}. Uptake of oxidized lipoproteins may trigger the macrophage to secrete cytokines, growth factors and other mediators that stimulate collagen secretion, promote smooth muscle proliferation and further increase LDL oxidation — all of which would increase the

mass of the plaque⁵. In most tissues such a response would be part of normal healing or scarring, but in the confined space of a coronary artery wall even a tiny scar is catastrophic.

Cigarette smoke oxidizes LDL and unmasks its binding to scavenger-cell receptors¹⁹. Ageing is accompanied by an accumulation of oxidized proteins²⁰. Hypertension increases collagen in arteries. Do scavenger receptors play a protective role in all of these processes? We can hope that studies such as those described here will, in time, deliver a simple unifying theory that explains how cholesterol, cigarette smoke, hypertension, ageing and inherited factors cause atherosclerosis. □

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LEVITATING GYROS

Good vibrations for physics

S. H. Salter

THE apparently counterintuitive behaviour of a gyro is conventionally explained in terms of the principle of conservation of angular momentum, which is itself a direct extension of the conservation of linear momentum. Both principles are reasonable and intellectually satisfactory. Both have led to many reliable machine designs. And the explanation gives the relationships between torque and angular velocity, but does not allow the generation of a single force without a reaction. Nevertheless, numerous determined inventors have set out to produce such a force. Several, most recently H. Hayasaka and S. Takeuchi (*Phys. Rev. Lett.* **63**, 2701–2704;

1989), have built gyro machines that behave in ways that are difficult to explain and which might support such claims. A successful machine would overturn the foundations of classical physics and could find practical applications in space travel.

Many of the devices use cams and linkages to induce combinations of forced precession in their flywheel rotors. The phenomena described by Hayasaka and Takeuchi, however, involve a much simpler mechanism — a plain gyro sitting on the pan of a laboratory balance with its rotor vertical (Fig. 1). They report an apparent loss of weight for one spin direction but no change in weight for the other (Fig. 2). The magnitude of the change is not large, only 11 mg for a 175-g rotor spinning at 13,000 revolutions per minute (r.p.m.), but the weight losses are repeatable and many times greater than the claimed accuracy (0.3 mg) of the balance used. The loss seems to be proportional to the first powers of rotor mass and angular velocity.

Hayasaka and Takeuchi have taken great care to eliminate many possible artefacts. The rotor is in an evacuated enclosure to prevent aerodynamic effects. The observations are not changed if the frame is inverted and the spin reversed to keep the spin vector the same. They are not affected if the positions of the gyro and the comparison weights are exchanged. Measurements are made as the rotor slows down with the electrical drive disconnected. They can be repeated in a magnetically screened enclosure. The results on an 'old-fashioned' swinging-beam chemical balance with standard comparison weights are very close to those on an electronic

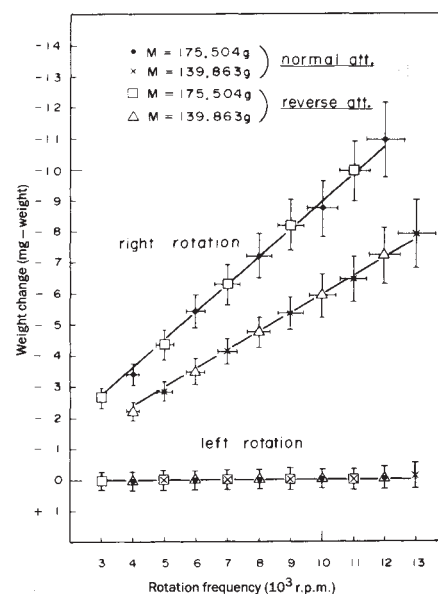


FIG. 2 The weight losses observed by Hayasaka and Takeuchi for the two directions of rotation. (From ref. 1.)

one using current in a coil for force feedback. The scatter of the observations is reasonably low and there would be little hesitation in accepting them in any less contentious field.

Note that, although the data in Fig. 1 allow convincing straight line fits, the projections of these straight lines do not meet at a point corresponding to zero weight change for zero speed: one would expect zero right-hand rotation to produce the same effect as zero left-hand rotation. Note also that the scatter of observations increases with the magnitude of the weight change. The scatter is about the same as the balance accuracy for small weight change and about four times greater for the maximum weight changes. Finally, the expression suggested for weight loss is unusual among the algebraic equations for gyro behaviour in that it uses the mass of the rotor times the *first power* of an 'equivalent radius', rather than its moment of inertia (a second-power function of radius).

A defender of the classical position is faced with two distinct problems. He or she must explain not only how there could be any effect for one direction of spin, but why there should be none for the other. It is to the credit of Hayasaka and Takeuchi that their paper includes observations that provide some clues — measurements of the vibration of the 140-g rotor at 13,000 r.p.m. It is possible to construct an argument to show that vibration in the gyro, compounded by nonlinearity in the weighing mechanisms (that is, forcing and response are not proportional), could lead to a misleading result.

In one spin direction the vibrational acceleration was 0.0995 g and in the other 0.0965 g. Not only are these values quite large — a tenth of g, the acceleration of gravity — but they are significantly

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FIG. 1 Hayasaka and Takeuchi's experiment, with a simple gyro sitting in the right-hand pan of a conventional chemical balance.

different (by 3 per cent) for the two directions of spin and "nearly the same" for the normal and inverted attitudes. We are not told whether the measurements are axial or radial, nor whether they are amplitude or root-mean-square values.

Neither do the authors give the mass of the static parts of the gyro's frame, or details of the suspension and sensors used in the measurements. But the argument can proceed with reasonable assumptions. Assume, for example, that the frame and the stator of the electric motor also weigh 140 g, that the acceleration measurements were made with the gyro package on a soft suspension with a light accelerometer and that the acceleration values were the amplitudes of sinusoidal signals. The amplitude of the vibrational force acting on the frame for 13,000-r.p.m. rotations would then be about 14 millinewtons. (Peak forces in a more complex vibration could be about three times greater still.) The reported weight change for the 140-g rotor at the same speed corresponds to a force of only 78 micronewtons, 180 times smaller.

On reflection, one can see that even good commercial ball bearings would give vibrations of this magnitude along the axis of the gyro, the sensitive direction of the balance: a simple sinusoidal deviation on the two tracks of the ballrace would produce an axial displacement in phase with the shaft revolution. With perfectly accurate balls, the maximum separation of the bearing tracks occurs when the high spots on each are opposite one another. The tracks would come together when the low spot of one was opposite the high spot of the other. Such movement would give a relative sinusoidal acceleration of the two parts with an amplitude of half the peak-to-peak error of the better track multiplied by the square of the rotation frequency. A track error with an axial component of only $1\text{ }\mu\text{m}$ rotating at 13,000 r.p.m. would produce a relative sinusoidal acceleration of 1.85 m s^{-2} . With comparable masses for the rotor and frame, the absolute acceleration of each would be 0.93 m s^{-2} or 0.095 g , almost exactly the values observed.

The designers of laboratory balances do not generally expect them to be subjected to active loads. Indeed, in most balance rooms strict precautions are taken to suppress building vibrations much less than one tenth of gravity. Only a brave dynamicist would predict what would happen when an excitation so much larger than the balance's resolution is injected into and then reflected back from a mechanism as complicated as a chemical balance. Even if the various spring and inertial parts of the balance were to behave in a linear fashion, the damping mechanism is unlikely to be truly linear. And although an electronic balance might seem mechanically simpler, there are still

plenty of possible sources of nonlinearity; for example, in the flexure stiffness of the leaf-springs, which is increased in tension and reduced in compression. And the null detector will have been chosen for its sensitivity and the stability of its null point but not necessarily for good linearity a long way from the null point. Excessive variations in the signal to the feedback circuit may well be clipped.

The nonlinearity of the balance's damping could be either symmetrical or asymmetrical. An asymmetric damping mechanism would give an obvious rectification of the vibration. But even a symmetric, nonlinear damping mechanism would rectify if the distribution of the velocity of the vibration was skew (Fig. 3). Some further thought about bearing manufacture suggests that some asymmetry of axial cam-error velocities is quite

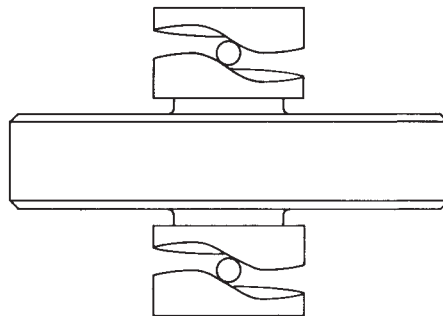


FIG. 3 A gross exaggeration of the asymmetric axial cam-slopes of the bearing tracks. Any nonlinear damping would rectify the resulting velocity to produce an incorrect weight reading.

likely. The spindle bearings in the machine tools used to grind bearings are often pre-loaded against their seatings by an elastic element working through a second thrust bearing. A high axial error in the main spindle bearing would force the workpiece into the grinding wheel faster than a low error would retract it. It is particularly interesting that Hayasaka and Takeuchi observed different vibration amplitudes for the two directions.

But why were the results unchanged on inverting the gyro? Bearings are sold with various grades of precision after a selective inspection process. If the upper and lower bearings used in an experiment are from the same batch it is likely that they will have been made on the same machine and will have been selected for the same tolerance band, so that they will have similarly asymmetric slopes. This could be tested by direct measurements of the velocity of the gyro's frame, for example by attaching it to the coil of an audio loudspeaker and statistically analysing the coil's output voltage.

Asymmetric cam-slopes would explain why the apparent weight losses would be independent of attitude and different for the two spin directions, but not why the loss for the left rotation would be zero. There are several other possible sources of

nonlinearity in the bearing mechanism, however. A high-frequency component of the axial acceleration might exceed 1 g so that the rotor might momentarily lift off the track like a car being driven too fast over a hump-backed bridge. The subsequent return would produce an abrupt spike of force. The deflection of the hertzian contacts between the balls and their tracks depends on load to the $2/3$ -power. The deflection of a single 2-mm ball between planes under a weight of 140 g is nearly $0.9\text{ }\mu\text{m}$. It may well be that several vibration mechanisms are operating and that, by chance, in one direction they cancel.

Defenders of the classical position can reasonably ask inventors of gyro thrust machines to produce equipment in which the alternating forces are not so very much larger than the claimed thrust effects. If this cannot be done, it is necessary to prove beyond doubt that the forcesensing apparatus is not affected by the alternating excitations. Such a test could easily be performed by attaching a dense non-ferrous mass to the central part of an ordinary low-power audio loudspeaker which would be substituted for the gyro. The speaker coil would be driven via an a.c.-coupled amplifier, preferably with a high output-impedance current-drive. It would be fed with waveforms such as low-mark/space-ratio pulse trains and asymmetric ramps to induce asymmetric velocities with absolutely no gyro behaviour. If this excitation produced any apparent change in the weight of the loudspeaker, the suitability of that force-detector for use in gyro thrust experiments would be brought into question. Hayasaka and Takeuchi could also do some interesting experiments by the rigid attachment of masses to their gyro frames, by inverting their present bearings in their seatings, by applying a variable axial preload and by fitting noisier bearings — perhaps some which have been deliberately abused.

It might be possible to reduce vibration by careful phasing of the high spots of a pair of bearings pre-loaded against one another. The relaxation of international tension may lead to the availability of missile-grade gyros which ought to be much quieter. Perhaps the ideal instrument would be a combination of a gyro, a moving coil vibration source and an accelerometer mounted on a force-sensitive platform designed for live loads. If the same weight losses can be produced with both quieter and noisier bearings and if the loudspeaker tests show that the balances used by Hayasaka and Takeuchi are not affected by vibration with asymmetric velocity, classical physics will be faced with a most interesting problem. □

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The calm before the quake?

Thomas H. Heaton

WITH the exception of Alaska, the Cascadia subduction zone, extending 1,200 km from northern California to Vancouver Island, is the largest tectonically active fault system in North America. Although it is widely accepted that the North American continental plate is converging at a rate of 2.5–4.5 cm yr⁻¹, modern seismic activity has been minimal along the Cascadia subduction zone. Are the Pacific oceanic plates sliding effortlessly below the continental margin? Or are the plates welded together, gradually accumulating elastic strain, to be released in another cataclysmic earthquake sometime in the coming centuries? There is geological evidence, much debated at a recent meeting*, that there have been six massive earthquakes (energy magnitude $M_w = 8.5$ –9.5; see box overleaf) in this region in the past 3,600 years, most recently sometime in 1680–90.

Several years ago, S. Hartzell and I argued that the Cascadia subduction zone might be locked, with slip occurring only during infrequent large earthquakes (*Science* **236**, 162–168; 1987). We concluded that the Cascadia subduction zone shares many characteristics with the Nankai Trough off southwestern Japan (convergence rate of 4 cm yr⁻¹), the site of $M_w = 8.1$ earthquakes in 1944 and 1946, and the southern Chilean subduction zone (convergence rate 8 cm yr⁻¹), the source of the catastrophic $M_w = 9.5$ earthquake in 1960.

But at the meeting, D. Byrne and L. Sykes (both at Lamont-Doherty Geological Observatory) argued that the accretionary wedge of the Cascadia subduction zone is too weak to support such large earthquakes. They suggested, rather, that the Cascadia subduction zone is similar to the Makran subduction zone of southern Iran which may be subducting by steady-state creep (no large earthquakes). Although an $M_w = 8.1$ earthquake occurred in the Makran region in 1945, Byrne and Sykes presented seismological evidence that this was a normal faulting earthquake that did not occur along the main plate boundary. However, L. Kulm (Oregon State University) pointed out that there is significant variation in the apparent properties of the continental margin along the Cascadia subduction zone, and that Eocene crystalline rocks are common in the continental margin.

Nearly ten years ago J. Savage and others (*J. geophys. Res.* **86**, 4929–4940; 1981) reported evidence that strain is

accumulating parallel to the direction of plate convergence. Additional studies since have corroborated this conclusion, with the largest strain rates (about 0.2 microstrain yr⁻¹) observed in coastal regions, including the area just north of the Mendocino triple junction (M. Lisowski and W. Prescott; US Geological Survey, Menlo Park). The relative rates of vertical deformation along the Oregon coast, over the past 50 years, as indicated by levelling data (M. Vincent and R. Weldon, University of Oregon; M. Richards, University of California, Berkeley), seems to be much greater than those inferred from the dating of uplifted Quaternary marine terraces. The observed historic strain and uplift data

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Ghost forest of western red cedar (*Thuja plicata* Donn.) protruding through a tidal marsh in coastal Washington state. The man to the left at the waterfront stands on roots that mark the present level of ghost-forest floor. According to recent geological and dendrochronological studies, the forest subsided into the intertidal zone during a great earthquake on the Cascadia subduction zone in about 1690.

were independently interpreted by Vincent and others, Lisowski and others, and H. Melosh (University of Arizona) as evidence that these strains, that accumulate over centuries, are released in great subduction earthquakes.

The most compelling evidence that great subduction earthquakes have occurred comes from stratigraphic studies of Holocene coastal lowlands. Since the initial report by B. Atwater (US Geological Survey, Seattle; *Science* **236**, 942–944; 1987) that coastal lowlands in Washington have repeatedly subsided in the past several thousand years, many others have found buried, abruptly subsided lowlands at coastal sites ranging from Vancouver Island to Humboldt Bay, California. Atwater reported that many stratigraphic features seen along the Cascadia subduction zone are similar to those observed in a recent study (unpublished) of coastal deposits resulting from coastal subsidence during the 1960 $M_w = 9.5$ Chilean earth-

quake. J. Bourgeois (University of Washington) reported that sand sheets deposited on lowlands of southern Chile by the tsunami wave from the 1960 earthquake resemble sand sheets that cover buried wetlands of coastal Washington and Oregon. The analysis by M. Reinhart (University of Washington) of the thickness distribution of these sand deposits from the last event in Washington is compatible with deposition associated with a 10-m tsunami, but not with a large storm surge.

Dating of the coastal subsidence is important not only for determining recurrence intervals for the inferred earthquakes, but also for learning the maximum coastal extent of individual events, as a guide to rupture length and earthquake magnitude. W. Grant (US Geological Survey, Seattle) cited 200 radiocarbon ages for buried lowlands in Washington, Oregon and northern California as evidence of episodic, sudden deformation of regional extent. Known episodes in the past 2,000 years occurred about 1,700 years ago (two episodes within several hundred years of one another, known for southern Washington and northern Oregon), 1,100 years ago (known for northern Oregon and northernmost Washington) and 300 years ago (known for northern California, Oregon and southern Washington).

The radiocarbon ages have too much error to show whether these episodes each consisted of a single great earthquake, or whether each episode comprises several lesser earthquakes. D. Yamaguchi (University of Colorado) obtained better precision by using ring-width patterns to date trees that died from subsiding into the intertidal zone during the most recent episode (about 300 years ago; see figure). The trees come from six sites scattered along a 90-km stretch of the southern Washington coast. Although most of the trees have lost their outermost rings to weathering, dates for the outermost preserved rings cluster in the 1670s and 1680s. The most recent great earthquake on the Washington part of the Cascadia subduction zone therefore seems to have occurred about 1690 — more than half a century after the founding of the Massachusetts colony.

Extraordinary geological events, perhaps coeval with great Cascadia earthquakes, have also occurred inland. R. Buckman (US Geological Survey) described a marine terrace 10 km west of Seattle that underwent 7 m of rapid uplift sometime in the past 1,700 years. A map by H. Gower and others (*US Geol. Surv. Misc. Invest. Map I-1613*, 1985) shows that the probable structure on which this uplift occurred extends through the centre of Seattle. P. Williams (Lawrence Berke-

Brian F. Atwater

*American Geophysical Union fall meeting, San Francisco, 4–8 December 1989.

ley Laboratory) reported that three huge landslides fell into Lake Washington, just east of Seattle, about 1,100 years ago, and that two others occurred there about 1,700 years ago. He suggested that the slides were triggered by great earthquakes on the Cascadia subduction zone.

The suggestion that great Cascadia subduction earthquakes occur has evolved in the past decade from hypothetical conjecture to a widely discussed model that seems to provide the best explanation for many observations. Yet, with the subduction zone quiet since Europeans arrived in northwestern America about 200 years ago, few of the region's 10 million people have become prepared for the minutes of strong ground shaking and the devastating tsunami that a great Cascadia earthquake would produce. Although basic scientific questions remain about the earthquake potential of the Cascadia subduction zone, many researchers feel that the evidence is sufficient to warrant updating building codes (particularly in Oregon) and tsunami evacuation plans to reflect the potential for great subduction earthquakes. □

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Earthquake magnitude scales

Charles Richter's original magnitude scale (usually called the local magnitude M_L), developed in the 1930s, was designed to measure the size of waves recorded in southern California for local earthquakes. To generalize the restrictive range of seismograms and earthquakes that could be quantified with the local magnitude scale, many other magnitude scales have been developed in the past 50 years. Surface-wave magnitude (M_s) measures the size of long-period surface waves recorded at large distances from earthquakes and it is one of the most popular magnitude scales in use today. It became clear in the 1970s that existing magnitude scales did not adequately represent the total energy of very large earthquakes. M_w is an energy magnitude scale obtained from estimates of the total kinetic energy radiated by an earthquake (H. Kanamori *J. geophys. Res.* 82, 2981–2987; 1977). It is defined to give values comparable to Richter's original magnitude scale for earthquakes as large as magnitude 6. For each unit increase in M_w , the total kinetic energy increases by a factor of 31.6. Therefore an earthquake of $M_w=9.0$ radiates about 1,000 times as much kinetic energy as the recent 17 October 1989 $M_w=7.0$ Loma Prieta earthquake in California. Unfortunately, the many different existing magnitude scales are generally all included together in the maddeningly vague term 'Richter Scale', which is popular with the press, but meaningless to a seismologist. T.H.H.

Colour consciousness in archaeology

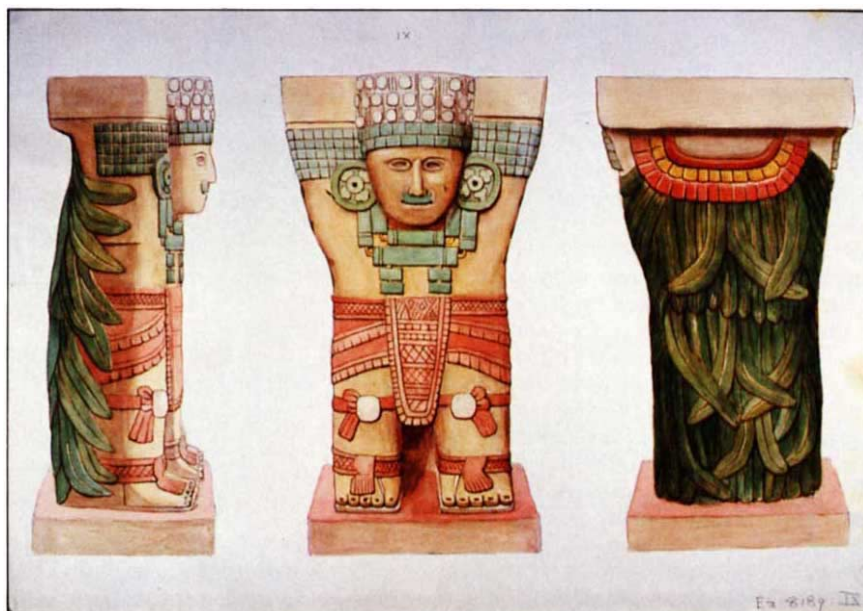
ADELA Catherine Breton (1849–1923), the painter of the picture reproduced below, has a special place in the history of Maya archaeology. In 13 visits to Mexico and Central America around the turn of the century, she photographed and painted the major prehispanic ruins and, at her death, bequeathed her collections to Bristol City Museum. The Museum has, for the first time, put the best of this material on display*.

What excites professional mayanists is the series of watercolour copies Breton made of wall paintings and architectural details, many of which are now damaged beyond repair. Her diaries describe how

sacred or precious, pertaining to worship and to the divine rights of kings.

In her lifetime Adela Breton was highly regarded by the founding fathers of Maya research, but her work was largely neglected in the 1960s and 1970s, when archaeology was attempting to be a science and its practitioners were barely speaking to the art historians. Today there is a happier relationship between dirt archaeologists, epigraphers and art historians, and I predict that many specialists will make their way to Bristol.

At the time when the Breton exhibition was opening, a disquieting report appeared in Vol. 11 of *The Pre-Columbian Art*



she searched out traces of pigment in cracks and crannies in order to produce original-colour renderings of sculptured monuments. The example illustrated here (a caryatid from the Upper Temple of the Jaguars at Chichén Itzá) may disconcert tourists used to the beauty of plain stone, but this colour is what the Maya artist meant us to see. Without it, one would not realize that the cape was made of green quetzal feathers and the necklace of jade.

To a Maya observer, details of costume and insignia gave precise information about a personage's nature (human or divine) and about his rank and status in the world. A decorated Maya facade was designed to be 'read' for its message as well as appreciated for its beauty, and in this system of visual communication colour-coding played a vital part. Red, for instance, was used for human figures; blue was reserved for gods and for things

Research Institute Newsletter on the damage done to Maya monuments by acid rain, algae, windblown dust and the ravages of mass tourism. This survey draws on a three-year investigation by Merle Robertson on behalf of the National Geographic Society. To quote from the summary: "there is not going to be any visible sculpture or painted buildings or any kind of buildings for that matter a 100 years from now. The paint will be gone long before that, in fact, it is almost all gone NOW". Already the world-famous Bonampak murals, after a generation of mistreatment, can be better appreciated in museum replicas than in the original temple, and Palenque was documented just in time. Meanwhile, Adela Breton's fine watercolours become increasingly precious records of Maya culture.

Warwick Bray

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*The Art of Ruins: Miss Adela Breton and the Temples of Mexico is at the City of Bristol Museum and Art Gallery, Queen's Road, Bristol, until 10 March.

Putting a twist in the tale

John Galloway

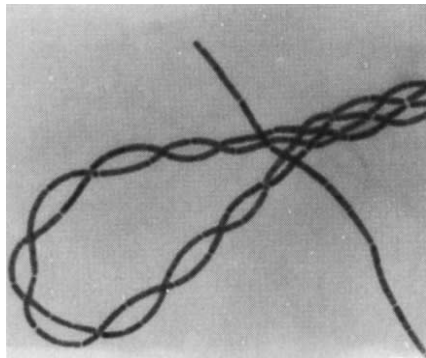
THE best idea that D'Arcy Thompson¹ contributed to biology was that biological forms are "fashioned by the direct action of physical forces operating during growth"². The idea is wrong (except in a rather trivial sense) if interpreted narrowly, but right if viewed more broadly. A recent paper by Mendelson and Thwaites³ returns to Thompson's theme. The paper is worth reading not simply because it does so, but also because the authors use a striking and unusual experimental model — the lytic-deficient mutations of the bacterium *Bacillus subtilis*.

In fact, Thompson's idea is true in more senses than one. Generally speaking, as Gould² among others has pointed out, physical forces are not the immediate causes of particular forms but rather determine the optimal structures at which evolution 'aims'. Neither the structures of animals and plants, nor evolution, can be understood except through a consideration of physical forces. And indeed, the notion of an animal or plant being 'well designed' for its physical environment is not comprehensible except through those considerations². Thompson was also right in a secondary, but at the same time more direct, sense. Within limits, an individual organism can change its structure to suit a changing environment, so physical forces must sometimes have direct influences on gene expression. Instances of this abound. One that is rather striking, and that is cited by Mendelson and Thwaites³, is provided by *Vibrio parahaemolyticus*⁴. This is a marine bacterium normally propelling itself with a single polar flagellum. An increase in viscosity of the liquid in which it swims leads to the bacterium growing additional lateral flagella to improve its swimming speed, and which it uses to swarm. It looks as though increasing viscous drag, possibly acting on the flagellum, has a direct activating effect on a gene (or genes).

Using the precept that structural and evolutionary principles are likely to be seen most clearly in simple forms, bacteria might be good models for studying the physics of form more generally. The small icosahedral viruses (see for example ref. 5), and the helical viruses, are the clearest examples of simple optimal structures and of how evolution achieves them. Bacteria, being larger and more complicated, ought to provide additional insights, particularly into the comparative necessity and sufficiency of genes for determining structure and what other influences need to be considered. It might be expected for instance that bacterial structure is more 'flexible' than viral structure, with a greater range of possibilities consistent

with the role the structure performs. From the work of Mendelson and Thwaites³ it looks as if this is so.

Bacillus subtilis is a common rod-shaped Gram-positive bacterium about 0.8 μm in diameter and a few micrometres in length. The cylindrical shape is maintained by a fairly stiff (though still elastic) tubular outer coat composed mainly of peptidoglycan and teichoic acid⁶. In general, such a shape readily lends itself to a helical mode of construction⁷, so apt comparisons might be made with cylindrical viruses, such as tobacco mosaic virus, or the filamentous bacterial viruses. The problem is that whereas viral capsids have been the subjects of intensive study by X-ray diffraction and electron microscopy, the coat of *B. subtilis* has not been described in nearly so much detail.



The long thread-like clones of *Bacillus subtilis*. The double-helical threads fold repeatedly to form unusual helical structures called 'macrobes', which twist either to the left or the right. In some conditional mutants the direction of twist is temperature dependent.

This is where the lytic-deficient mutations come in⁸. Growth results not in increased numbers of individual bacteria but in long thread-like clones which may have an unusual double-helical morphology. These double-helical threads fold repeatedly to form helical, multicellular 'macrofibres' ('macrobes'; see figure) that are structurally analogous to twisted textile yarns⁹.

Macrobes behave in some ways like more orthodox multicellular organisms. They have the advantage, however, of a relatively simple physical structure that is susceptible to precise geometrical analysis — a helical hierarchy, some of the parameters of which can be measured fairly readily and others inferred. A macrobe is therefore an amplifier of the structure-determining features of the individual cells. It is plausible to presume that these features are aspects of the architecture of the cell wall, and that it therefore has a helical architecture. This is in part confirmed by observing cinematically the rate

of twisting of the ends of growing clones⁹; the range of twisting seen in different macrobe strains indicates a pitch angle of up to about 8° from the axis of the bacterial filaments for the arrangement of the structural material.

The best observations are of screw sense. Some strains are left-handed, others right. Others again are 'conditional' mutants — they may be either left or right, and the degree of twist can vary continuously between left-handed and right-handed extremes depending on environmental factors, such as temperature. Twist varies in a roughly linear way with respect to the temperature at which the culture is grown, passing through zero twist at about 40 °C. Right-hand clones are produced at lower temperatures, left-hand at higher ones⁹. Clones transferred from one temperature to another gradually assume the twist characteristics of that temperature including the switching of screw sense.

Macrobes growing right-handedly at one extreme of temperature apparently retain a memory of a brief exposure to the other — left-hand inducing — temperature extreme¹⁰. This manifests itself as a short-lived reflection of screw sense some time later. Presumably this is a result of enzyme synthesis. If so, because the reverse (left to right) does not happen, it seems that a protein is needed for left-handed structures but not right-handed. (Similar asymmetric behaviour is seen in the early development of snails; right-handedness in *Limnaea peregra* needs a protein, left-handedness apparently does not¹¹.)

It seems that peptidoglycan is important in twisting behaviour, because untwisting precedes dissolution of macrobes following the application of lysozyme. But even better supported is the idea that the unusual amino acid D-alanine, which is present in interglycan linkages, is crucial in determining twistedness. If D-alanine is made available externally (it is usually synthesized from L-alanine by the bacteria) a complicated concentration-dependent effect is seen. At some concentrations a left-handed helix is produced, at others a right-handed one. The antibiotic D-cycloserine counters the effects of D-alanine in whichever direction so that the hand reverts towards being under the direct control of the gene¹².

The helix offers biology a very popular geometry because organisms are largely variations on the two themes of fibres and hollow tubes⁷, both of which readily lend themselves to helical modes of construction. The coats of helical viruses self-assemble, which offers the simplest building mode, but this cannot be true of the bacterial coat of *B. subtilis*, which must depend on some sort of 'machine' to put the strings of peptidoglycan into the coat. Among other things, it is unlikely that

self-assembly would offer the possibility of both left- and right-helical hands. Better comparisons can be made with the fibre patterns in plant cell walls; for instance, helical windings in cotton fibre cells follow both left-handed or right-handed paths and often abruptly change hands¹³. Neville¹⁴ has suggested that there is an interplay between molecular self-assembly into helicoidal structures and mechanical reorientation due to growth forces. And it is worth noting that Lloyd¹⁵ has suggested that the central role in the formation of these helical patterns is played by microtubules.

In the past few years there has been decided progress in uncovering how genes control patterns of early development (the segmented pattern of insect embryos for example), and how the genetic information is converted into a sort of relief map of morphogenic gradient¹⁶. But there is more than one way to skin a cat and more than one way to control shape. Handedness is a fundamental quality of biological form, as D'Arcy Thompson appreciated, MEMBRANE BIOLOGY

and Mendelson's group has found an interesting experimental system in which to look at it. □

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The shadow and the substance

Walter Gratzer

THE injunction that one should waste no pure thoughts on impure proteins, often imputed to Kornberg, was (according to the man himself) actually voiced by Racker. The aphorism retained its pith until molecular genetics finally revealed how protein chemistry might be done without any protein. No one who has laboured to purify refractory and uncooperative proteins from membranes will fail to be stirred by the amount of information that Kopito *et al.*, writing in *Cell* (59, 927–937; 1989), have garnered about the nature, structure and properties of the neuronal membrane anion-exchange protein without even reaching for a bottle of detergent.

It has been known for some years that the well-characterized anion exchanger of the human red cell — the preponderant membrane protein, band 3, or in the new terminology, AE1 — has immunologically detectable counterparts in other tissues, including brain, but almost nothing has until now been discovered about their properties. Band 3 itself has been sequenced by way of the gene. First the chicken and mouse sequences were established, and in 1988 Tanner and his colleagues (*Biochem. J.* **256**, 703–712; 1988) finally got the human sequence out. It has now been independently determined by Lux *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **86**, 9089–9093; 1989), and the two DNA sequences agree — remarkably, for it is a sizeable protein — down to a single base in the coding sequence. The two separate

domains, recognized by earlier chemical studies, are apparent: there is a largely membrane-associated N-terminal half and a cytoplasmic piece. The evidently α -helical membrane-spanning segments can be discerned, and it seems that the chain makes 14 passes through the lipid bilayer, with small loops of varying length on the outer cell surface. The intracellular domain has binding sites for various red-cell proteins, most importantly ankyrin, the main link between the membrane skeletal network and the membrane. At the extracellular surface there is a binding site, containing at least one lysine, for a family of stilbene disulphonate anion-transport inhibitors.

From Passow's laboratory in Frankfurt came the news that messenger RNA from mouse spleen, or indeed band 3 complementary RNA, when injected into *Xenopus* oocytes leads to the appearance of band 3 in the membrane. It is functional and subject to inhibition by the stilbene disulphonate inhibitor, DIDS (Bartel *et al.* *EMBO J.* **8**, 3601–3609; 1989). Garcia and Lodish (*J. biol. Chem.* **264**, 19607–19713; 1989) obtained a similar result with the messenger for the human protein. Both groups set out to identify the lysine(s) at the inhibitor binding site. It had been confidently predicted that a sequence-conserved lysine (558 in the mouse, equivalent to 539 in man), or possibly another lysine, three residues along in the sequence (missing, however, in the chicken), would be the spot. Garcia

and Lodish mutated lysine 539, but to no effect; it must then, they inferred, be the other lysine, if earlier data in the literature were correct. Bartel *et al.*, however, went a step further with their mouse clone, and replaced both. The DIDS still bound and inhibited, although covalent attachment, which requires a reactive lysine side-chain, was expunged. Clearly the site is near; but, as so often, nature is more complex than the biochemist desires.

Back then to Kopito and the neurons: a red-cell cDNA probe picked out a mouse genomic restriction fragment, which was revealed as representing a new band-3-like protein (AE3). The whole sequence was found in two overlapping cDNA clones and after that all was plain sailing. First the sequence, when determined, turned out to be quite closely related to that of red-cell band 3 (AE1) and a corresponding kidney protein sequence (AE2), obtained by a similar procedure in Lodish's laboratory a year ago. In the C-terminal, anion-transporting part of the molecule all three sequences are closely related: the similarity between AE3 and AE1 amounts to some 80 per cent of the amino acids, with 70 per cent identity. Hydrophobicity plots show superimposable systems of peaks and valleys; the pattern of bilayer-traversing segments stands out.

Next, Kopito and his colleagues demonstrated that transfected cultured (COS) cells expressed the AE3 with considerable efficiency. The cells that showed expression were also positive by immunofluorescence, and their cytoplasmic pH was measured with a fluorescent indicator. Anions were transported through the membrane, and when the external medium contained carbonate-bicarbonate, the cytoplasm became acidified. The inhibitor, DIDS, did its thing. Moreover deletion of the entire N-terminal half of the sequence (645 amino acids) from the DNA still led to a functional protein, although the acidification that it brought about was a little less. A survey by immunofluorescence indicated that, in nature, AE3 is probably confined to neurons.

So more or less without seeing the protein, Kopito *et al.* have discovered that neurons possess an acidifying anion exchanger, the transport properties of which they have characterized; that it will home in on and insert itself into the membrane without benefit of a leader sequence; that the C-terminal half of the molecule suffices to express its function; and that the activity is very probably regulated by the interaction of the business end with the pendant cytoplasmic domain. And more than that one scarcely needs to know. □

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A SQUID's loss of coherence

Ian Moss

QUANTUM cosmologists, accepting that the natural scale of events in quantum gravity is a mere 10^{-33} cm (the Planck length), have become accustomed to the idea that laboratory tests of their theories are unlikely to be forthcoming. Thus, the idea now put forward by Ellis, Mohanty and Nanopoulos¹ that SQUIDS, small superconducting magnetometers, might be noticeably affected by Planck-scale gravitational 'wormholes' is bound to be taken up with interest.

The special property of a SQUID — properly a superconducting quantum interference device — that is relevant here is that it displays coherent quantum phenomena despite its macroscopic size. A more familiar example of coherence is found when light is passed through a pair of slits and projected onto a screen: with low intensities, an interference pattern is seen, representing the relative phase of the light waves passing through each slit. Increase the intensity of the source and the pattern fades, because many bunches of photons arrive at the screen with random relative phases. This loss of phase coherence is typical of macroscopic systems in which the interactions between numerous particles obliterate the phase information.

For SQUIDS, in which the phase is that of the electrons passing through a resistive barrier, the low level of dissipation allows it to remain in a prepared coherent state on timescales of 10^{-4} s. Ellis and colleagues suggest that this time is sufficiently long for an additional mechanism that destroys coherence — quantum wormholes — to become apparent.

In any theory of quantum gravity, quantum fluctuations are expected to arise in both space and time. A theory of quantum wormholes has been developed to describe these fluctuations. A wormhole represents the creation of a 'baby universe', a self-contained piece of space that is disconnected from the rest of the Universe and owes its existence to a quantum fluctuation. Wormholes have another important property: they connect distant parts of space and time, rather like bridges in the geometry of space.

Any piece of space would be riddled with these wormholes, but their tiny size makes them far too small to be seen. Their only effect is to introduce many stochastic parameters into the world, which are the quantum numbers of the baby universes. These parameters modify all the fundamental constants of nature. They also give a means for losing coherence by scrambling of the quantum phases.

The strength of the effect that these wormholes can have on a macroscopic

device is determined by a single parameter, λ . Unfortunately, the theory of quantum wormholes is too immature for us to calculate λ reliably. Stephen Hawking has pioneered calculations of the kind needed^{2,3}, and finds that λ should have a value of the order of either one or zero: one corresponds to a large effect, so that zero is the only reasonable value.

But Sidney Coleman⁴ has improved on Hawking's methods by including the effects of not just one, but many wormholes. The result is that the baby-universe parameters are driven to definite values (see L. F. Abbott's News and Views article⁵). Coleman calls this the "big fix". If he is correct, the constants of nature are fixed and quantum wormholes cannot destroy coherence.

But if Coleman is wrong, as Ellis and colleagues assume for their calculations, the gravitational scrambling of phase may be possible. They suggest, somewhat arbitrarily, that the parameter λ is given by the ratio of the electron mass (10^{-31} g) to the scale mass, or Planck mass, for quantum gravity, 10^{-5} g — a value which is not unreasonable on dimensional grounds, but which has no firm physical basis. This

value is sufficiently small for the wormholes not to influence most physical phenomena. But in the coherent state of the SQUID, its effect becomes multiplied by the number of electrons in the SQUID, around 10^{19} , so that there is a measurable effect: Ellis and colleagues suggest that coherence should be lost on a timescale of 1,000 s. To observe the loss of coherence, the authors suggest firing a beam of polarized neutrons through the centre of the SQUID (a ring-shaped device) and observing the decay of the oscillatory interference pattern they produce owing to the device's magnetic field. The difficulty would be to distinguish the weak wormhole effect from the stronger conventional effects.

It may be a little premature to be making such detailed predictions about wormholes, given the current state of theory. On the other hand, it is possible that the authors have made a lucky guess and we could be on the verge of measuring something truly astonishing. □

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FUEL CELLS

Miniaturized electrochemistry

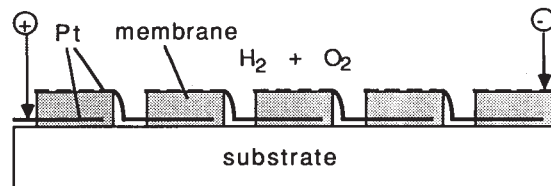
Thomas E. Mallouk

OVER the past decade, electrochemists have turned their attention towards smaller and smaller devices. Electrochemical cells made with micrometre- and nanometre-size components have physical characteristics which differ from those of their macroscopic relatives, and they have been widely studied because of potential applications as chemical sensors and molecular electronic devices. On page 547 of this issue¹, C. K. Dyer describes a solid-state fuel cell, based on the oxygen-hydrogen reaction, that is less than a micrometre across. Although its current output is only low (around $100 \mu\text{A cm}^{-2}$, compared with 100 mA cm^{-2} as available from conventional fuel cells under similar conditions²), its simple design and small size make it appropriate for use in series arrays to power microchips (see figure).

Conventional fuel cells, which were essential, for example, to the Apollo programme, use the combustion of hydrogen or methane to generate small voltages. To do this, they are constructed in the manner of an electrochemical battery,

with the oxidation of hydrogen at one electrode releasing electrons and reduction of oxygen at the other mopping them up. The unexpected novelty of Dyer's cell is that it develops an unusually large voltage in mixtures of hydrogen and oxygen.

Both the magnitude and the polarity of the open-circuit voltage are hard to explain. If the two half-cell reactions (oxi-



A series of thin-film fuel cells, after Dyer's design, operating in a mixture of hydrogen and oxygen.

dation and reduction) are kinetically facile at both of the platinum electrodes that constitute the cell, it is hard to see how a voltage can be generated between the electrodes from gas mixtures. Unless one invokes Maxwell's demon to partition the reagents between the electrodes, one is forced to suppose that hydrogen gettering, or some similar mechanism, at the exposed outer electrode promotes hydro-

gen oxidation there, or that oxidation is suppressed at the membrane-covered inner electrode. Whether other physical phenomena, uniquely associated with the small size of the device, can affect the transport of reactants and cause spontaneous polarization of the cell remains to be investigated. The answer may lead to devices with improved performance.

The thin-film fuel cell is only one of several recently described microscopic electrochemical devices of various capabilities that can be fabricated by lithographic techniques. Extremely small dimensions allow the use of relatively poor electrical conductors (pseudoboehmite in the case of Dyer's fuel cell) as components without excessive ohmic losses. The use of microlithography also opens up the possibility of integrating electrochemistry with electronics; in the resulting composite systems an electrochemical cell interfaces an electronic device with the chemical 'real world', receiving or transmitting chemical signals. For sensor applications, an additional multiplex advantage is gained, because components that are selective for different chemical signals can be applied to an array of transistor gates or photodiodes to be read simultaneously.

In an interesting variation on this theme, Parce *et al.*³ have shown that a silicon chip can be fabricated into a 'microphysiometer' by etching an array of 50- μm square wells in the silicon and covering them with thin insulating layers of SiO_2 and Si_3N_4 . A glass cover slip placed over the array traps a small number of living cells within each well. Because living cells produce CO_2 and lactate, the change of pH within the wells is related to their metabolic rate. The Si_3N_4 overlayer is pH sensitive, and the device therefore acts as a microscopic pH meter, reading the transient metabolic response of as few as a thousand cells to various chemical and biochemical stimuli. It is likely to be useful for *in vitro* screening of therapeutic drugs, and detection of biochemicals, viruses, and products released by one cell that affect a second cell.

Aviram and co-workers^{4,5} have published experimental and theoretical papers on what may be the ultimate goal in integrated electrochemical/microelectronic devices — single electroactive molecules that act as switching elements in electronic circuits. Molecular-orbital calculations indicate that molecules consisting of orthogonal conducting polymer strands, linked by a spiroalkane bridge, could form the basis of logic gates. An electric field of the order of 10^6 V cm^{-1} applied parallel to the bridge would cause an electron to be transferred between strands, causing them to switch between conductive and nonconductive states⁵.

In related experiments, a catechol/orthoquinone molecule was prepared as a self-assembling monolayer on a gold

surface, and interrogated with the tip of a scanning tunnelling microscope (STM)⁶. The molecule is orientated in such a way that the catechol moiety is bound to the gold, through a thioether group, and the orthoquinone group is nearer the STM tip. When the gold is positive with respect to the STM tip, a direct current flows, presumably because the catechol is oxidized and the quinone is reduced; the resulting semiquinone-semiquinone diradical is thought to be a good electrical conductor. In line with this interpretation, no current flows when the polarity is reversed. Similar diode-like behaviour has been observed using an STM tip to address individual atomic defects on the surface of silicon^{6,7}.

The wiring and interconnection of individual molecules is obviously the greatest challenge in this work, and chemical rules which may be useful are now beginning to emerge. Laibinis *et al.*⁸ have shown, for example, that 'hard' and 'soft' acid-base interactions between chemical functional groups and metal or metal-oxide surfaces can be used to direct molecules to bind in specific regions of a microelectronic array. With an interdigitated array of oxidized aluminium and gold microwires, they showed that alkanethiols and alkylcarboxylic acids dissolved in the same solution bind exclusively the gold and aluminium surfaces, respectively. One can easily envisage molecules with thiol and carboxylate functionalities at either end which could bridge between dissimilar metal contacts in a microelectronic structure. True molecular electronics based on such structures would require a practical technique (nonexistent at present) for arranging patterns of these wires on the scale of molecular dimensions.

Although Dyer's thin-film fuel cell produces small currents, it is possible that an improved understanding of its operation will lead to successful high-power applications, as in electric vehicle propulsion. The fuel cell is at present interesting as a power source for low-current applications, for example in remote sensing and microelectronics. One can now imagine integrated microelectrochemical devices, based on this emerging technology, that incorporate the elements of energy conversion, chemical sensing and molecular computing. $\square$

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Light engineering

A RADIOTHERAPIST treating a cancer will try to maximize the radiation dose to the cancer while minimizing it for the surrounding tissue. This can be achieved by aiming the beam successively from many directions, intersecting at the cancer. Daedalus is now generalizing this strategy. He claims that a complete set of beams, each of a properly computed intensity and direction, could impose any desired pattern of radiation intensity on the interior of the object at which they were aimed.

The process is simply the converse of X-ray tomography. An X-ray beam projected through an object emerges with an intensity that sums the X-ray absorption densities along its path. A tomographic scanner records the emergent beam intensity for every possible line of intersection with the object, so as to compute the density at each point inside it. Daedalus's inverse tomograph projects a pre-computed beam intensity along every possible line of intersection with its target, so as to deliver a specific summed radiation dose to each point inside it.

While the inverse-tomograph may well sharpen radiotherapy, Daedalus wants to apply it to engineering. Aimed under computer control at a tank of radiation-sensitive liquid monomer, its X-ray or ultraviolet beam could 'sculpt' a chosen shape in radiation density. The monomer would photopolymerize into a perfect copy of that shape, which could then be pulled out of the tank. Shapes impossible to mould, with arbitrarily complex re-entrants, holes, or internal detail, could be created cheaply and flexibly by pure software, and could be updated and modified at will.

More subtly still, the inverse tomograph could make objects whose physical properties varied from point to point. A graded radiation pattern could lightly crosslink the polymer to a pliant rubber at one point, while setting it firmly to rigid polymer at another. Crash helmets with a hard skin but soft interior, luggage with a rigid frame but yielding exterior, shock-proof mountings with a complex pattern of solid, elastic and damping regions, could all be formed in one operation from a single material.

Daedalus is even designing a combined inverse tomograph and bubble chamber, to produce variable density foamed plastics. A volatile liquid monomer under reduced pressure might boil and foam in highly irradiated regions. Plastic components could thus be made hard and dense at the surface or in regions subject to heavy loads, but foam-light elsewhere. The engineering dream of a complete structure free of joints and fastenings, as light as possible and just strong enough at each point to bear the local load, would be a reality at last.

David Jones

Models of the Great Red Spot

SIR—We note that the experiments of Sommeria *et al.*¹ and the numerical simulations of Marcus² are not acceptable models of Jupiter's Great Red Spot (GRS) because their approach does not meet the necessary conditions.

Necessary conditions for an adequate model of the GRS-like vortices include: (1) cyclonic–anticyclonic asymmetry — almost all large, long-lived vortices in the jovian and saturnian atmospheres are anticyclones (including the GRS vortex)^{3,4}; (2) large radii, a , of the vortices (the characteristic length of the velocity gradient) relative to the barotropic Rossby–Obukhov radius, r_R (for a vertically homogeneous fluid) or the baroclinic Rossby radius, r_i (for an inhomogeneous fluid); for example, for the GRS, $a \approx 5,000$ km (ref. 5), $r_i = 1,000$ – $1,500$ km (refs 3,5), and $a \gg r_i$. (Here $r_R = (gH)^{1/2} \Omega \sin \varphi$, where g is the acceleration due to gravity, H the thickness of an atmosphere, and φ is the latitude; $r_i \approx (1/5-1/4) r_R$ in real atmosphere.)

Conditions (1) and (2) correspond to the 'intermediate geostrophic' (IG) regime of the Rossby vortex motion ($a > r_R$). Both of them were satisfied in our experiments⁶⁻⁸ (see ref. 3 for review). In refs 1 and 2, a method of vortex production that allows generation only of cyclones and eliminates generation of anticyclones was used, not satisfying condition (1). In addition, the model fluid used has no free surface (it is contained between rigid lids), thus corresponding to a regime with $r_R \rightarrow \infty$ and, hence $a \ll r_R$, r_i ('quasi-geostrophic' (QG) approximation, $a < r_R$), not satisfying condition (2).

We produced^{13,6,8} a long-lived monopole IG Rossby anticyclonic soliton and so obtained a laboratory model of the jovian GRS driven by smooth counterstreaming fluid flows. We also showed that the long-lived Rossby soliton exists only when it has a sufficiently large amplitude and involves captured particles, that is, the Rossby soliton is a real vortex. Regarding cyclones, we showed that they were only generated by the counterstreaming cyclonic flows under the condition of extremely strong velocity shear, similar to conditions necessary for the existence of jovian cyclonic 'barges' (14° N).

The vortex Rossby soliton concept allows us to interpret satisfactorily the principal properties of the GRS: its anticyclonic polarity, its size, its rotation speed and its steady westward drift, its generation by counterstreaming flows (existing in jovian atmosphere) and its uniqueness along the whole perimeter of Jupiter. In agreement with this concept, there is a large, long-lived vortex in Jupiter's Northern Hemisphere (19° N) which corresponds to the GRS of the

Southern Hemisphere (22° S): like the GRS, it is an anticyclone, it drifts westward with a speed of 2.5 m s^{-1} and has a size $a > r_i$. Because of its physical similarity to the GRS it is called the Little Red Spot⁹.

An essential part of our results is represented incorrectly in ref. 1: we did observe the merging of vortex Rossby solitons under their mutual collisions. This phenomenon is in good agreement with the observed properties of the GRS and with the theory of the vortex (not 'purely wave') Rossby soliton; it also agrees with the computer work⁴. This phenomenon is in qualitative disagreement with the first ('purely wave') soliton theory of the GRS¹⁰; the latter uses the QG approximation ($a < r_i$) and does not agree with the GRS observations.

Our results are not affected by viscosity because, under the conditions of our experiments^{6,7}, the characteristic decay time of the IG Rossby anticyclone is about 20–25 s (ref. 11); hence, it is an order of magnitude greater than the vortex turnover time (≈ 2 s). The point is that, in the presence of a free surface, the viscous lifetime (τ_{visc}) of the IG Rossby vortex is much greater than the Ekman time (τ_E), because¹¹⁻¹³ $\tau_{\text{visc}} = \tau_E (1+F)$, where the Froude number, $F = a^2/r^2$, is much larger than unity for the IG regime. We predict that Neptune's Great Dark spot discovered recently¹⁴ is an anticyclone.

However, we must say that independent of the above, the experiments of Sommeria *et al.*¹ seem to be very impressive from a hydrodynamical point of view.

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MARCUS AND SOMMERIA *ET AL.* REPLY—The theory and experiments of Antipov *et al.*⁶⁻⁸ are in the intermediate geostrophic regime, and ours^{1,2} are in the quasi-geostrophic. Both IG and QG are special cases of single-layer shallow-water theory but the approximations are mutually exclusive, and the dynamics of long-lived vortices are different for the two regimes. Thus the issue is whether the GRS is QG or IG — we argue it is QG based on Voyager observations.

The distinction between IG and QG depends on the size of the baroclinic (inner) Rossby radius r_i . For scales of motion larger than r_i , the IG rather than the QG approximation is appropriate. Estimations of r_i near the GRS range from 500 to 5,000 km; hence, because the GRS is 12,000 km by 26,000 km, several

authors⁴ conclude the GRS must be IG. Voyager data, however, show that the GRS velocity is mostly in a thin, large diameter, annular region. The correct length scale to compare with r_i is the half-width of this annulus, approximately 2,000 km, which is the characteristic scale l over which the velocity varies. A careful comparison of Voyager data with numerical solutions of full shallow-water equations yields^{15,16} $2,000 \text{ km} \leq r_i \leq 3,000 \text{ km}$; hence, the QG approximation for the GRS is justified. The numerical solutions also show that the potential vorticity q can be approximated to within 15% by the QG expression $q = Q[(f + \omega)^{-1} \nabla \cdot (f^{-1} \nabla gh) - gh(fr_i)^{-2}]$, where ω and Q are the vorticity and potential vorticity of the zonal velocity, h the height of the upper surface of the vortex not including the zonal component, f the Coriolis parameter and g the effective gravity. Our simulations show that QG vortex dynamics are *insensitive* to the value of r_i . Therefore, experiments with a rigid lid ($r_i = \infty$) simulate GRS dynamics.

We showed¹² that a large QG vortex can form from the turbulent merging of smaller vortices. The resultant large vortex (which is not a soliton) has approximately uniform q (which forces $l \approx r_i$) and a quiet interior with v and vorticity increasing exponentially until they peak in an annular strip of thickness $2r_i$ at the vortex's edge. This is a very good description of the GRS. By contrast, an IG Rossby soliton is gaussian with vorticity and angular velocity peaked at its centre⁴.

Antipov *et al.* emphasize that the GRS is anticyclonic, as in IG theory, whereas QG vortices are cyclonic or anticyclonic. Their use of IG theory to explain the GRS's anticyclonicity is not compelling because another explanation is then needed for the majority of jovian vortices: most of the > 100 vortices observed by Voyager have $l \leq R \leq 1,300$ km, where R is the vortex radius. Even if r_i were as small as 1,300 km, these vortices would be QG — yet 90% are anticyclonic¹⁷. Thus, physics beyond the shallow-water approximations is needed to explain the anticyclonicity.

Antipov *et al.* argue that westward drifts of the GRS (-3.5 m s^{-1}) and Little Red Spot (-2.5 m s^{-1}) support IG-soliton theory because QG vortices remain stationary with respect to the local zonal flow, whereas IG solitons drift westward at the Rossby drift velocity: $-4.6/(1,000 \text{ km})^2 \text{ m s}^{-1}$ at the latitude of the GRS. Drift speeds of jovian vortices are measured with respect to System III coordinates, but there are uncertainties of at least $\pm 5 \text{ m s}^{-1}$ in determining the velocity of the deep zonal flow with respect to System III. Thus, the vortex drift speeds are all zero within the observational uncertainty. Williams and Wilson¹⁸ appeal to the uncertain zonal velocity to salvage their IG soliton model of the White Oval BC, which drifts eastward at 4 m s^{-1} . Note

that with $r_i = 1,500$ km, the IG-soliton GRS drift speed would be 10 m s^{-1} which is outside $-3.5 \pm 5 \text{ m s}^{-1}$.

Our experiments and simulations show that in a turbulent QG shear flow, vortices grow and are sustained by merging. Similarly, 24 mergers of vortex pairs in the same zone were observed by Voyager¹⁷. Antipov *et al.* also observed merging, but merging *per se* occurs in a wide variety of flows. The mergers Antipov *et al.* observed were not in a turbulent shear flow and not in the regime where they observed a persistent solitary vortex (refs 3,6–8 describe merging of transient vortices generated by a rotating disk in a laminar flow). The striking feature of the vortices on Jupiter and in our experiments is their persistence in the presence of turbulence. In a rapidly rotating tank, there is fast dissipation. The vortices of Antipov *et al.* survive longer than the Ekman friction time because the fluid layer is locally thicker at the vortex. In the surrounding thin fluid layer, however, the turbulence is strongly damped by Ekman friction. Thus, the vortices of Antipov *et al.* are laminar.

Although we disagree with Antipov *et al.*'s identification of the GRS as an IG soliton, we are fascinated by their experiments and would like to understand them better. Measurements of vortex size and shape as a function of fluid depth and shear strength would be helpful.

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Ubihomologous homology usage

SIR—The body of scientific ideas often grows faster than the vocabulary available for its expression, but the imprecise use of the word 'homology' in molecular biochemistry to mean 'sequence similarity' is unnecessary and can be obstructive because the two concepts conveyed by the single word are related¹.

The tendency in biology to assign homology to various generalizable associations manifests itself further in immunology, where the word 'homologous' takes on at least two additional meanings. In one sense, 'homologous' is used, largely in molecular immunology but also in other areas, to describe the relationship of an antibody with its naturally eliciting antigen, and stands in contrast to heterologous. By this definition, the combining site of anti-bovine serum albumin antibody is homologous with bovine serum albumin and heterologous with bovine myoglobin. Thus, 'homology' implies a causal physiological link, usually in association with a high degree of structural complementarity. (It does not refer to the structural complementarity itself.)

Unfortunately, this is not the only meaning of homology in immunology. 'Homologous', in its broad sense, also refers to the relationship of an antigen to an immunogenic host of the same species. Bovine serum albumin (BSA) is thus homologous to cows but heterologous to rabbits. Is rabbit anti-bovine serum albumin antibody, then, homologous or heterologous to BSA? Many immunologists would say the answer depends upon the context in which the question is asked, and that the intention would be readily understood by anyone working in the field.

The terminological ambiguity increases in more complicated cases. Suppose an immunologist is investigating two chemically distinct forms of bovine serum albumin, BSA₁ and BSA₂. He immunizes a rabbit with BSA₁ and finds that some of the antibodies elicited react with BSA₂. What are the relationships of BSA₁ and BSA₂ to these antibodies? Some molecular immunologists might say BSA₁ is the homologous antigen whereas BSA₂ is a heterologous antigen, even though BSA₁ and BSA₂ are from the same species and, in this more general biological sense, are both heterologous to the antibodies.

Alternatively, suppose a cow is immunized with rabbit serum albumin and the antibodies found to react with BSA. Such results have often been observed². Is BSA in this instance a heterologous antigen? And what of the relation between rabbit anti-BSA antibody and the antibody it elicits in a cow? Would the eliciting and elicited antibodies in this case be referred to as heterologous? As antibodies produced by different species, they are. As an antibody–antigen pair, they are not.

The potential for confusion in the description of antibody–antigen reactions also arises in that of cellular receptor–antigen interactions.

Although immunologists generally recognize the terms as they apply in their own field, for the sake of consistency throughout immunology and biology, the terminology needs to be clarified.

We propose that the use of the words homologous and heterologous in biology be reserved for the indication of suggestion of common phylogenetic ancestry or the lack thereof. Relationships among and within species may be aptly described by such terms as allogeneic, xenogeneic and syngeneic as in xenogeneic graft, allogeneic antigen (or simply 'alloantigen'). As for the relationship between antibody and antigen or between receptor and antigen, we suggest the introduction of two new terms: homoligous (homo-LYE-gous) and heteroligous (hetero-LYE-gous), the former referring to an eliciting elicited-ligand–receptor pair and the latter to a pair lacking the causal link and describable in terms of degree of cross-reactivity.

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All shook up

SIR—The recent experiments¹ (see ref. 2), in which a spinning gyroscope apparently loses weight, have an obvious flaw: anyone who has seen components travelling on a vibration-feed conveyer will have doubts about weighing vibrating objects.

Gyroscopes contain spinning material and so must vibrate. Given that gyroscope bearings are imperfect, the vibration could consist of a linear movement followed by a return movement — at a slightly different speed. This asymmetric vibration will interact with the damping mechanisms in the weighing device. For example, mechanical friction would be less for the faster movement. So pivot bearings in a balance could exert a thrust on the gyroscope being weighed. The mystery is no more.

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■ See also S.H. Salter's fuller description of this effect on page 509 of this issue.

The stone that put the stars to flight

Edward Harrison

Absolute or Relative Motion? A Study from a Machian Point of View of the Discovery and the Structure of Dynamic Theories. Volume 1: The Discovery of Dynamics. By Julian B. Barbour. Cambridge University Press: 1989. Pp. 746. £60, \$95.

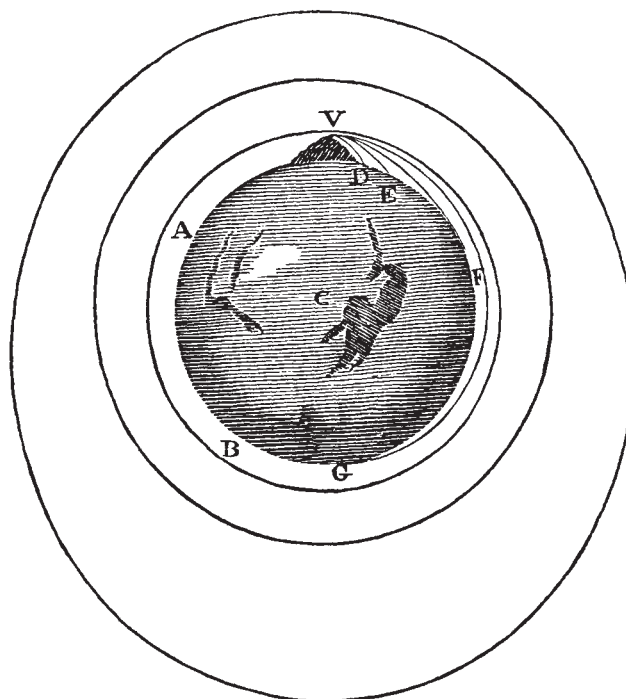
My sense of weariness at the sight of this large, erudite work vanished on reading the preface and introduction. Here is a call in the darkness to all natural philosophers yearning for insight into the ultimate mystery — the unity of the physical universe.

The aim of this study, declares the author, is to trace the evolution of the main strands in the history of dynamics, terminating in the theory of general relativity. The first volume surveys this enormous subject in technical detail from antiquity to the cartesian and newtonian systems of the seventeenth century that secured the scientific revolution. Students of physics rarely comprehend the scope of the historical background to such simple statements as $F = ma$. (The second volume, *The Frame of the World*, yet to be published, covers the period from Newton to Einstein.)

What holds the attention is the unfolding of the struggle to understand at a fundamental level the nature of space, time and motion, motivated by the dream that here lie the secrets of a rational system of the world. Each change in the concept of motion discloses a new universe. As a scientist, Barbour does not shun the mathematical and scientific details that so many historians seem to think are inessential. He sees the history of science as a many-stranded tapestry of interconnected ideas into which he weaves a colourful pattern of machian concepts. I have no intention of taking issue with this novel infusion of Mach's principle; in fact I think we must be grateful to Barbour for drawing our attention to the machian overtones in the history of dynamics and for giving helpful interpretations of the nebulous ideas embodied in Mach's principle. Some students may prefer a less embroidered history, as in *The Fabric of the Heavens* (by Toulmin and Goodfield; Harper and Row), and a straighter account of dynamics, as in *Newtonian Mechanics* (by French; Norton).

The Discovery of Dynamics begins with the atomists, who held that space is an invisible receptacle existing independently of matter. But Aristotle opposed this view, holding that space without matter is nothing, it has no extension, and hence atoms separated by voids do not exist. Barbour shows that the atomist-

aristotelian dichotomy affects all aspects of the history of science. After a long discussion on hellenistic astronomy comes a short chapter on mediaeval developments, followed by the copernican



"For a stone . . . the greater the velocity with which it is projected, the further it goes before it falls to earth . . . till at last, exceeding the limits of the earth [it] will pass into space." Illustration and quotation from *The Systems of the World*, by Isaac Newton.

system, the galilean geometrization of motion, and a keplerian world dominated by the Sun and an abhorrence of centreless infinite space. The second half of the book is devoted mainly to the works of Descartes and Newton. Descartes, whose scientific work is not fully appreciated by English-speaking people, introduced key concepts, including the materialism (God creates but does not thereafter control the world) essential for the mathematization and mechanization of a world of matter in motion. But whatever Descartes said, Newton said the opposite. Descartes's aristotelian ideas of space permeated with matter and of forces acting by direct contact were opposed by Newton's atomist ideas on matter moving in empty space and of forces acting at a distance. Newton stressed the reality of independent space. By asserting that absolute space exists in

its own right, and that uniform motion is relative but accelerated motion is absolute, he prepared the way for his celebrated laws of motion.

"Where there is no matter there is no space", protested Leibniz, echoing Aristotle. All motion, uniform and accelerated, is "relative to the stars" and not to an empty nothing, declared Berkeley. Unlike his critics, who had no effective theory of dynamics and gravity, Newton performed experiments (with a spinning bucket of water) to demonstrate what he meant. Barbour discusses relative and absolute motion, and relative and absolute space, arguing that Newton's first law cannot provide the true grounds of knowledge. In my opinion, it depends

on whether unbounded space-time is regarded as physical or nonphysical. Barbour may be right in terms of outmoded ideas of space; in terms of geodesics in the theory of general relativity, the first law (and second law) has a secure physical meaning and makes perfect sense.

Before the rise of field theories, when science was concerned with the activity of material bodies, Ernst Mach (1838–1916) stated that the sole task of science is to establish relations between observed phenomena. He rejected as irrelevant whatever could not be directly observed, including absolute space, absolute motion, the atomic theory and, later, the theory of relativity. All motion, he said, is relative to the distant stars. "For me, only rela-

tive motions exist" and "I can see, in this regard, no distinction between rotation and translation". The inertia of a body arises from the distribution of the "masses of the universe". Mach's down-to-earth philosophy appealed to many scientists before matter vanished into an evanescence of fields, and space and time became active participants in the physical world.

Einstein referred to the cosmic origin of inertia as Mach's principle. The prospect of a machian unity in physics inspired Einstein in his search for a relativity theory in which the special class of inertial observers is widened by means of the equivalence principle to include the more general class of free-falling observers in gravitational fields. But contrary to Einstein's initial expectations, the general theory did not unambiguously embody Mach's principle, and the surviving

machian vestige is the enigmatic cosmological constant that may or may not link the atom and the cosmos.

Other promising prospects of unifying the physical universe have emerged in more recent years, and many cosmologists think that Mach's principle has joined the bulging rag-bag of discarded cosmological ideas. But, as Barbour shows, Mach's aspiration expresses an undying desire to discover a rational unity. In a supermachian form (as I have argued elsewhere), the principle reaches out to Whitehead's doctrine of internal relations, in which all identities are implicate within one another.

Is space absolute or relative? Is it real and independent of matter? My own guide is to consider the modern space-time continuum: it decomposes into space

and time peculiar to observers in relative motion; it relates to nothing external and hence is absolute; it is physically real and exists in its own right; and its dynamic curvatures propagate and are the source of further curvature (black holes may consist only of gravitational waves). When we appreciate the physical richness of the modern space-time continuum, the machian debate becomes of historical interest, and what remains are the fascinating supermachian hopes concerning the ultimate goal of cosmology. Presumably, some of this will be discussed by Barbour in his second volume. □

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Field tests

G. J. Jellis

Biological Control of Microbial Plant Pathogens. By R. Campbell. *Cambridge University Press: 1989. Pp. 218. Hbk £27.50, \$55; pbk £9.95, \$17.95.*

THE control of diseases on crop plants continues to be a pressing need throughout the world, whether to ensure a crop comes to fruition and produces an acceptable yield, or to produce a higher-quality product, free from blemishes and capable of being stored for long periods. For centuries, farmers (albeit often unconsciously) have been using methods for containing some of the worst ravages of disease — crop rotation, use of fertilizers and the selection of resistant varieties, for example. Such techniques can be grouped under the heading of biological control. R. J. Cook, one of the leading exponents of the subject, defines biological control as "the use of organisms, genes, or gene products to regulate a pathogen". More commonly, however, the term is restricted to the narrower concept of manipulating microbial interactions with a pathogen or host to keep the inoculum density of the pathogen below an economic threshold, to retard or exclude infection or to induce host resistance. Although this concept is not new, it has come very much to the fore in the current 'green' climate as a non-chemical (seen by the layman as environment-friendly) way of controlling plant diseases.

With such considerable interest, and the consequent increase in research funding, Campbell's book, designed for undergraduates and graduate students, is a welcome addition to the literature. It provides a good introduction to the subject, developing the theme in a manner both interesting and easy to follow. After

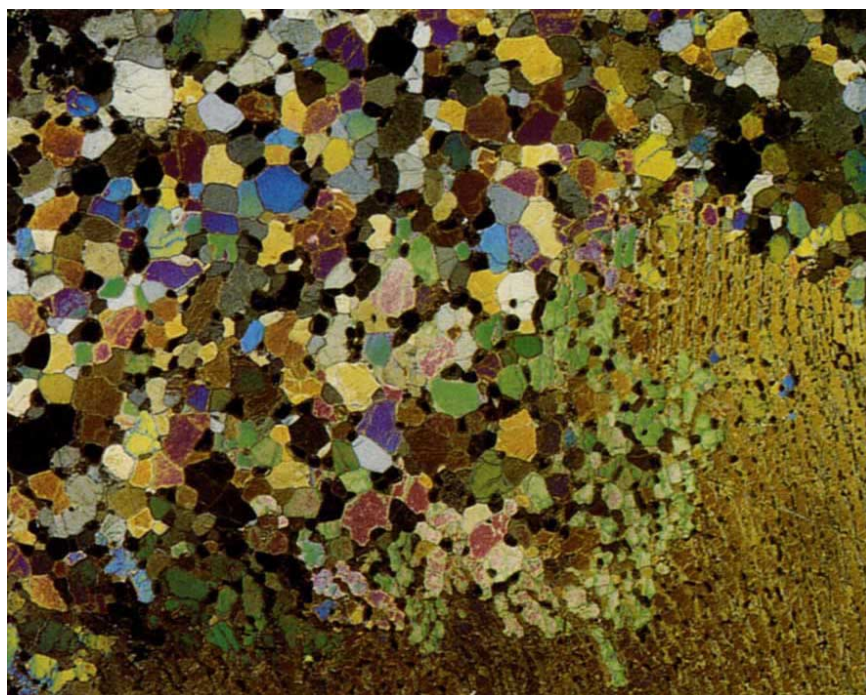
a few hours' pleasant reading, the average undergraduate reader would have a sound grasp of the concepts and a broad view of the state of the science. The book is also designed so that those who wish to learn about a particular aspect of biological control can do so, with the aid of a comprehensive subject index, expanded indices of pathogens and antagonists, a reference list and a glossary of terms.

As a plant pathologist with a particular interest in disease control but not a specialist in biological control in its narrow sense, I was delighted to find that,

throughout the book, Campbell considers biological control within the general framework of control strategies. But he does not avoid the problems, as well as the potential, of the approach. After describing some of the exciting experiments that have been done on leaf, stem, root, fruit and flower diseases, he brings the reader back down to earth in his concluding remarks to each chapter. Although biological control has much to offer, in general, the breakthrough from the closed system to the field has yet to come. Biological control also has its risks — one only has to think about some past mistakes in insect control such as the unsuccessful introduction of cane toads in Australia to curb the sugar-cane beetle. Both toad and beetle are now problems in Queensland.

Another appealing feature is the way Campbell describes the development of a product, from the basic science required to understand the mechanisms of biological control through to testing and patenting. This form of presentation should stimulate the next generation of plant pathologists to think of fresh approaches and, perhaps more immediately important, to find ways of implementing, in the field, control measures already developed in the laboratory. □

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Igneous art — volcanic rocks frequently contain inclusions (xenoliths) of different origin and composition. The photograph shows the complex metamorphosed structure of a mantle xenolith from Lake Bullenmerri in eastern Australia, as viewed through crossed-polarizing filters. The lamellar golden region is augite, a mineral of the clinopyroxene group, which has recrystallized elsewhere as a mosaic of polygonal augite, bronzite and garnet (black). The picture is reproduced from *Intraplate Volcanism in Eastern Australia and New Zealand*, by R.W. Johnson, published by Cambridge University Press, price £50, \$85.

Heroes and villains

Marian Stamp Dawkins

Animal Revolution: Changing Attitudes Towards Speciesism. By Richard D. Ryder. *Basil Blackwell: 1989. Pp.385. £17.50, \$24.95*

RICHARD Ryder has the distinction of having introduced a new word — speciesism — into the English language. In 1975, he used it to describe “the widespread discrimination that is practiced by man against other species, and to draw a parallel between it and racism” (*Victims of Science*, p.16). In his latest book, *Animal Revolution*, he describes the history of this idea from the ancient Egyptians to the present day.

Ryder gives an unashamedly personal view of the way humans treat animals. For the first half of the book, the fact that Ryder has strong views of his own adds to rather than detracts from the book's impact. He gives spice to his history by describing his heroes, such as St Neot, who was evidently one of the earliest hunt saboteurs, and villains such as Robert Baden-Powell, who described pig-sticking or hog-hunting as “manly and tip-top” (p.134). The reader can quite easily take the first ten chapters as one man's view of history, without necessarily agreeing with every word. Only the odd gratuitous swipe at the Royal Society for the Protection of Cruelty to Animals (RSPCA) mars an otherwise fascinating narrative, full of gems and nuggets of information. There are some excellent tales, too, such as Queen Victoria's behind-the-scenes support of the 1876 legislation on animal experiments and a somewhat spine-chilling quotation from George Bernard Shaw (p.132). Shaw wrote provocatively: “No sportsman wants to kill the fox or the pheasant as I want to kill him when I see him doing it.”

In turning from history to the present day, however, Ryder's own views become rather more intrusive. There is a fairly large section on the internal squabbles that took place within the RSPCA and his own role in them. This is ‘dirty linen’ history of a not very illuminating sort. The RSPCA is an important animal-welfare organization, but seen against the backdrop of the history of human attitudes to non-human animals, its private difficulties and what Ryder himself did to resolve them are surely not worth reporting in such detail.

There then follow substantial chapters on three key issues of animal welfare — wildlife conservation, animal experimentation and farming. Here, too, Ryder's views are intrusive to the point of distortion.

He pulls no punches about scientists and vivisectionists and their motives: “once they have been ‘bloodied’ they have a psychological vested interest in continuing their trade” (p.253). Nor does he conceal his contempt for many scientists and veterinarians and what they do to animals.

In describing modern farming methods, Ryder marshals some disturbing statistics about current practices, but he gives the impression that the issues are far more clear-cut than they really are. Many people will agree with him that there is a lot that should be changed about the way animals are kept on farms, but there are difficulties with abandoning current practices — such as the cannibalism and feather-pecking that are such a worrying feature of some commercial free-range and perchery systems for laying hens. By not even mentioning such problems and making it sound as though ‘alternatives’ to intensive systems are always, without question, better for animal welfare, Ryder weakens the very case he is trying to make.

It is, however, in the chapter called “Violence and the Animal Liberation Front” that Ryder is perhaps at his most controversial. He argues that violence against front members is actually more prevalent than violence by them and that to call them terrorists is a deliberate slur. As evidence that animal liberationists are in danger because of their beliefs, he cites cases of hunt saboteurs being attacked by huntsmen, the sinking of the Greenpeace flagship *Rainbow Warrior* and the murder of Dian Fossey.

Throughout the book, Ryder re-affirms his belief that speciesism is a moral wrong on a par with racism. His position is, as he describes it (p.8), the “startlingly simple” one that “whatever is morally wrong in the human case is probably wrong in the non-human case as well”. I found it disappointing that some of the serious and thoughtful critics of this view, such as Bernard Williams, are not mentioned. Anti-speciesism is an important idea but, paradoxically, the case for it could have been made more persuasively if more time had been given to discussing the genuine difficulties with it.

The way humans treat non-human animals is a matter of great importance, but to many people issues such as whether we should eat animals or do experiments on them are not “startlingly simple” but difficult in the extreme. One man's view of these matters, which is what Ryder's book is, will appeal to some, but probably fail to convince those for whom the moral issues are more complex. □

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Mixed metaphor

Sandra Panem

Shattered Mirrors: Our Search for Identity and Community in the AIDS Era. By Monroe E. Price. *Harvard University Press: 1989 Pp. 159. \$19.95, £15.95.*

DESPITE the global scope of AIDS, the analytical response to the epidemic has been in many ways a highly nationalistic and peculiarly American phenomenon. AIDS has as frequently been used as a vehicle to expose problems in US society as it has been to discuss the particular problems of the disease. The “American interpretation” of AIDS reflects the early emergence of the epidemic in the United States, the global dominance of the US biomedical establishment, and the predominant place that AIDS has had on the US socio-political agenda. During the past decade, AIDS has become a metaphor for a troubled American society.

Monroe Price's *Shattered Mirrors* is the newest entry in this parochial genre. Price's comments are focused through the distinctive American lens of the constitutional scholar. In a highly readable, and mercifully terse essay, the dilemmas raised by AIDS are seen to derive from the most basic tenants of US law. Price, Dean of the Benjamin N. Cardozo School of Law at Yeshiva University, clearly understands and documents what others know only instinctively.

AIDS is the first national text in a great while that even purports to address the propriety of adult behaviour . . . [there] . . . is a new focus on what the individual does, not just the heroic individual, or the researcher in the laboratory, but the ordinary person, the parent, the single adult, the adolescent child. All of a sudden, in the national consciousness, what the individual does is meaningful in terms of something larger, something connected to the fate of the community.

Price's interest seems to lie in the changing balance between policies that protect society at the expense of individual liberty and those that favour individualism. He cogently explains why this choice is especially hard in the United States, bolstering his argument with extensive reference to case and common law.

Students of the AIDS epidemic will find issues in Price's book that have not been raised before. Yet even when treading on all-too-familiar ground, Price brings an interesting interpretation. Take, for example, his handling of a complaint commonly heard early in the epidemic — that had the first afflicted group been more mainstream, the response to AIDS would have been very different:

Ours is, for better or for worse, a society that presumes, indeed thrives on inequities that arise not out of the denial of opportunity itself

but out of the differences in the way opportunity is seized. We know that the Constitution does not mean that every person will fare equally well. Yet, when we evaluate a course of government action — at least according to constitutional traditions — we must ask whether a higher level of scrutiny ought to be exercised because of the very nature of the risk groups affected by the AIDS crisis. If AIDS were a disease that struck only a racial minority, we would have the machinery to see if, within it, there was careless regulation that fulfilled discriminatory impulses rather than proper legislative needs. But the group first affected by government policy concerning AIDS — gays — is not defined by race. Yet it is the kind of group where the wisdom of Justice Stone is instantly relevant: Those at risk of obtaining AIDS are subject to the kind of "prejudice against discrete and insular minorities" that tends to affect the operation of political process in a manner contrary to our basic values (13). The consequence of being so disfavored a group is the kind of prejudice, as John Hart Ely has put it in *Democracy and Distrust*, that provides the natural majority with "a common motive to invade the rights of other citizens" (14).

Unlike many books concerned with the social implications of AIDS, Price's comments are most provocative for their implications for the future. If Price is correct, a sea change has occurred. Those interested in understanding the increasingly conservative tone of social thought will be rewarded by this book. □

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New in paperback

■ *Immune Responses, Virus Infections and Disease*, edited by N. J. Dimmock and P. D. Minor is based on a 1988 symposium of the Society of General Microbiology. It is available in paperback from Oxford University Press, price £18.

■ *Tectonics of Suspect Terranes* by D. G. Howell is the third volume in a series of four. Published in 1989, the series is available in paperback. Chapman and Hall, Vol. 3, price £17.50, \$35.

■ *Evolutionary Dynamics of a Natural Population* by B. R. Grant and P. R. Grant has recently been issued in paperback. Published by the University of Chicago Press, price \$24.95, £19.95.

■ *The Solid State: From Superconductors to Superalloys* by A. Guinier and R. Jullien. This translation from the French text is published by Oxford University Press, £15, \$29.95.

■ *Introduction to Metamorphic Textures and Microstructures* by A. J. Barker has recently become available in paperback. Published by Blackie in Britain, £13.95; Chapman and Hall in the United States, \$29.95.

In paperback

■ *Nuclear Power Development: Prospects in the 1990s* by S. M. Nealey, is a publication from the Battelle Press, 505 King Avenue, Columbus, Ohio 43201, USA, price \$18.95.

■ *Field Testing Genetically Modified Organisms* from the National Academy Press provides a practical guide for researchers, environmental organizations and policy-makers. Price \$19.95.

Cause and effect

John B. Whittow

Population and Disaster. Edited by John I. Clarke, Peter Curson, S. L. Kayastha and Prithvish Nag. Basil Blackwell: 1989. Pp. 292. £40.

It is 25 years since Gilbert F. White's *Natural Hazards, Local, National, Global* outlined the basic paradigm that has underpinned subsequent disaster research, namely a knowledge of the degree of occupancy of risk zones by people whose perceptions of hazard need to be tested in order both to predict their behavioural

that half the case studies in this volume involve the developing countries. One of the editors sets the tone by recognizing how "disasters have the ability to strip away much of the veneer of human life to reveal the basic patterns of human activity and behaviour".

Such revelations are often clouded when they are published by being presented in a disjointed form. This is not so in the present volume, which is a well-integrated and tightly structured collection of essays by an international group of geographers. Moreover, the mass of new information that the editors have produced conveys to me a hope that the malthusian doctrine of a catastrophe-maintained balance between population and resources need no longer

prevail if we can learn from past mistakes. Disasters of human causation, whether they be in China (chapter 10), Uganda (chapter 12), Chernobyl (chapter 13), the Gaza Strip (chapter 17) or South Africa (chapter 18) are capable of resolution by political and economic means, and even some of those induced by a combination of man and nature need not be as catastrophic as they sometimes are. For example, stricter building standards in the Soviet Union, or in the urban fabrics of Managua (chapter 3) and Mexico City (chapter 5),

would dramatically reduce the number of earthquake casualties, whereas a cessation of hostilities in Ethiopia (chapter 8) and the Sudan (chapter 9) could, together with a return to a better-balanced subsistence economy, improve the chances of the starving millions in these countries, notwithstanding the continuing drought.

All these studies give valuable insights into the widely varying problems of risk-zone populations, and some offer blueprints for future survival. In addition, there are chapters that provide a much broader appraisal by analysing the impact of wars and disease on global mortality rates, including a topical review of the threat presented by AIDS.

There is little doubt that this latest contribution to the few books which give a balanced account of the demographic consequences of both natural and human-induced disasters has succeeded in achieving its aim. Both the editors and the authors are to be congratulated on their lucid writing, their informative references and good-quality diagrams. This impressive volume should stimulate much rethinking by planners and policy makers at national and international levels. □

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IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Palestinian women carrying food are told by an Israeli soldier that they are breaking the curfew. Gaza Strip, January 1988.

responses and to ascertain the effectiveness of public policies in predicting and selecting the optimum damage-reducing adjustments. Foremost among those who have attempted to find answers to the questions posed by disaster research are in the North American hazards school of Ian Burton, Robert Kates and Kenneth Hewitt. But although these authors' investigations have been carried out throughout the world, they have almost always had the viewpoint of the industrialized Western nations.

Population and Disaster, by contrast, although adopting a similar framework, is written from a somewhat different perspective in so far as seven of its twenty-one authors come from developing countries. Indeed, it was the pressure brought to bear by Professor Kayastha, after his investigations of the Bhopal disaster in India (chapter 14), that led ultimately to the publication of this latest volume in the Special Series of the Institute of British Geographers.

Disasters that affect cities and transport links in the developed world are invariably newsworthy events, but famines, epidemics, wars and other catastrophes in developing countries receive far less media coverage. To rid us of our parochialism, therefore, one must welcome the fact

The formation of patterns in non-equilibrium growth

Eshel Ben-Jacob & Peter Garik

Crystal growth under non-equilibrium conditions can give rise to complex patterns which are generically similar to those found in processes such as viscous fingering, aggregation and electrochemical deposition. Recent theoretical understanding focuses on the interplay between the macroscopic driving force associated with the phase transition and the microscopic interfacial dynamics.

THE formation of patterns and shapes in the natural world has long been a source of fascination. Examples are many and varied, ranging from the growth of a snowflake to the aggregation of a soot particle, from oil recovery by fluid injection to solidification of metals, and from the formation of a coral reef to cell differentiation during embryonic development. The challenge is to understand the spontaneous formation of patterns from a disordered environment¹⁻⁴. Is each of these diverse patterns the result of unique causes and effects, or are there unifying underlying principles? The past few years have seen the emergence of a rough outline of such principles. These developments offer the hope that a unified theoretical framework can be formulated to provide a coherent picture of pattern formation.

The cornerstone of these developments is the recognition that growth patterns may be determined by the interplay between microscopic interfacial dynamics and external macroscopic ('thermodynamic') forces. Most research has focused on systems in which the macroscopic dynamics are determined by a diffusion field. For such systems, the patterns that form may be grouped into a small number of typical 'essential shapes' or morphologies, observed in different systems and over many length scales (from metres to micrometres). Some examples of these (faceted⁵, dendritic⁴, dense-branching⁶ and fractal⁷⁻⁹) are shown in Fig. 1.

The transfer of information from the microscopic to the macroscopic level is self-evident in the growth of a snowflake¹⁰, where the sixfold symmetry of the underlying ice crystal lattice is manifest in the dendritic branches of the flake. (In the formation of real snowflakes, however, other factors such as latent heat also play a role.) How is the macroscopic form influenced by microscopic effects in this way? One can obtain a qualitative understanding by considering the dynamics of non-equilibrium growth. In a system out of equilibrium a stable phase will propagate into an unstable or metastable one. In the formation of snowflakes, for example, the stable solid phase propagates into the unstable supersaturated water-vapour phase. The rate of growth of the stable phase is limited by a diffusion process, in this case the diffusion of water molecules from the gas phase into the crystal. In this process, the kinetics of diffusion tend to drive the system towards the formation of 'decorated' and irregular shapes. Diffusion kinetics determine the macroscopic approach towards equilibrium, and influence the structures created on many length scales. By contrast, the microscopic dynamics occurring at the interface (determined by surface tension, surface kinetics and anisotropy; see Box A) are associated with microscopic length scales and are influenced by molecular-scale symmetries. The final interfacial pattern results from the interaction between the microscopic and macroscopic levels. For example, if the system is sufficiently far from equilibrium, the interface can advance so rapidly that the stable phase does not have time to reach its lowest-energy state on the microscopic level, and a metastable microstructure results. The growth of quasicrystals is an example of such a process.

Box A: Microscopic interfacial dynamics

Surface tension reflects the microscopic forces between atoms (or molecules) at an interface^{4,5}. It lowers the temperature, T_i , of the curved parts of the interface according to:

$$T_i = T_m - \left(\frac{L}{C_p} \right) d_0 \kappa$$

Here L is the latent heat of the liquid-solid transition, C_p is the heat capacity of the liquid at constant pressure, T_m is the melting temperature of a flat interface, κ is the local curvature of the interface, and d_0 is the capillary length (proportional to the surface tension), typically of the order of nanometres. This equation (the Gibbs-Thompson relation) is a statement of local equilibrium for a curved interface. At local equilibrium, the free energy of the melt and the solid are the same, and there is no driving force either for melting or for solidification. For the interface to advance, there must be a degree of undercooling. We assume a linear relationship between interfacial (normal) velocity v_n and undercooling:

$$T_i = T_m - \left(\frac{L}{C_p} \right) d_0 \kappa - \left(\frac{L}{C_p} \right) \beta_0 v_n$$

The last term is meant to phenomenologically embody the microscopic dynamics of surface kinetics¹⁵, although experimentally the relationship seems to be more complicated⁴⁸.

The effects of surface tension and surface kinetics appear (with a different functional form) in almost all non-equilibrium growth processes. For example, in the Hele-Shaw cell the pressure along the interface is given by^{6,26}

$$P_i = P_a - d_0 \kappa - \beta_0 v_n$$

Here P_a is the applied pressure. The 'surface kinetics' term arises from the excess pressure needed to overcome the frictional drag of the fluid against the cell plates.

The third player in the game is anisotropy¹⁵. The average bonding energy between atoms at the interface depends on the orientation of the interface. Hence, it costs different amounts of energy to bend the interface in different orientations. The functional dependence is characteristic of the specific system. A tractable two-dimensional form is obtained by substituting d_0 for $d_0(1 - d_1 \cos(m\theta))$, where d_1 is the magnitude of the m -fold anisotropy and θ is the angle between some fixed direction in space and the direction normal to the interface. Surface kinetics are also a function of θ : an atom will attach itself to the interface at different rates, depending on the surface orientation, leading to a similar dependence on θ .

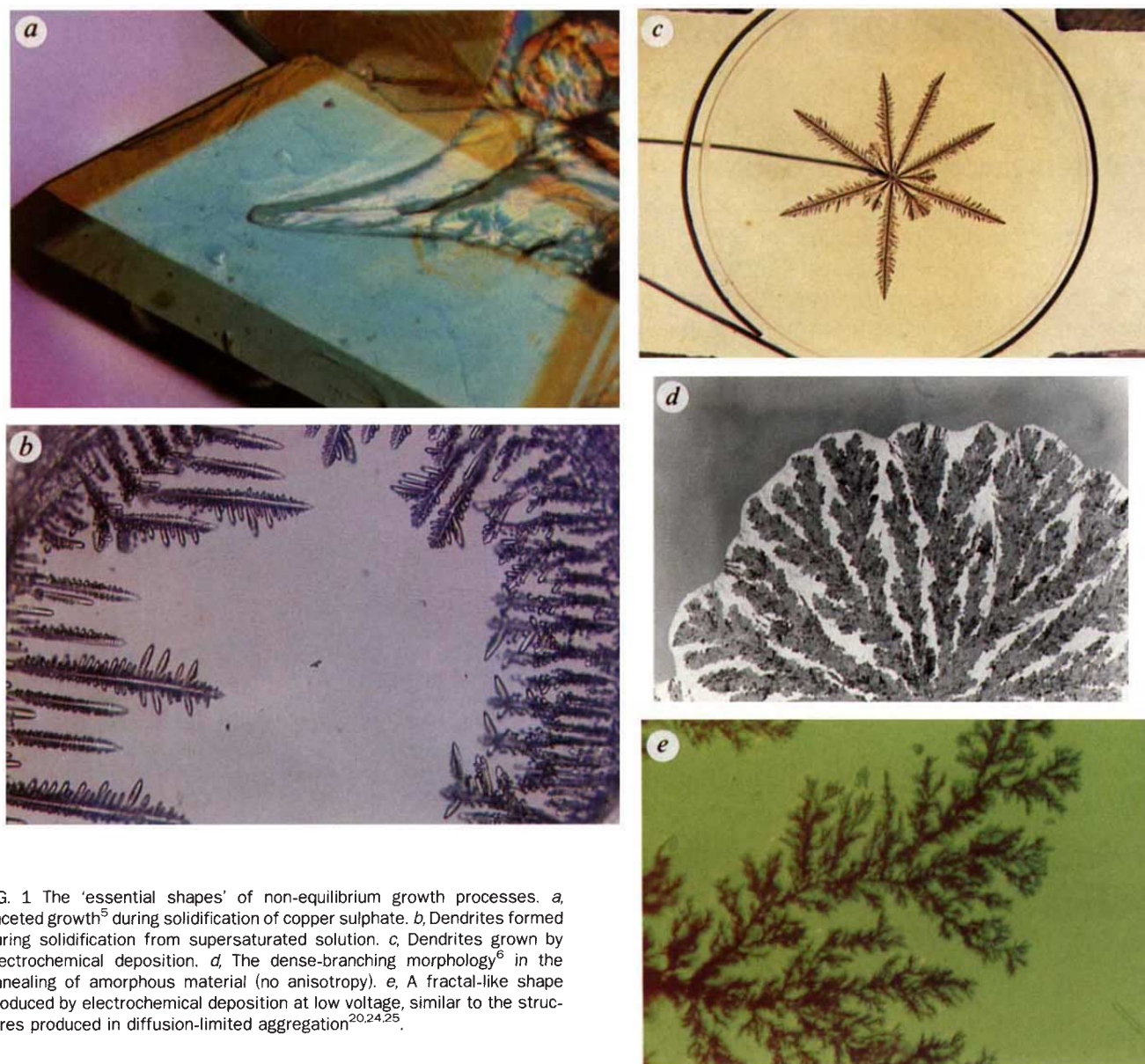


FIG. 1 The 'essential shapes' of non-equilibrium growth processes. *a*, Faceted growth⁵ during solidification of copper sulphate. *b*, Dendrites formed during solidification from supersaturated solution. *c*, Dendrites grown by electrochemical deposition. *d*, The dense-branching morphology⁶ in the annealing of amorphous material (no anisotropy). *e*, A fractal-like shape produced by electrochemical deposition at low voltage, similar to the structures produced in diffusion-limited aggregation^{20,24,25}.

The selection problem for dendritic growth

The principal problem that we try to resolve here is how microscopic dynamics, operative on the scale of ångströms, are amplified to such an extent that in a system out of equilibrium they control the macroscopic shape on a scale of centimetres. The natural inclination, however, is to strive for theories of growth that emphasize macroscopic dynamics and relegate the microscopic dynamics to a subsequent refinement; this was how the theory of dendritic growth initially evolved. Ivantsov showed¹¹ that for a solid forming from an undercooled melt, the propagating crystal front is parabolic when the process is controlled by heat diffusion alone (that is, when surface tension and surface kinetics are neglected). Both the parabolic shape of the tip and the predicted constant velocity are consistent with observations of dendritic growth. However, Ivantsov's solution specifies only the product of the dendrite tip's radius of curvature and its velocity; it cannot predict either one alone. Does this mean that dendrites with different tip curvatures and corresponding tip velocities, given by Ivantsov's criterion, coexist at a specified undercooling? Glicksman *et al.*¹² demonstrated that, for a given undercooling, only one dendrite (that is, one tip

velocity and radius of curvature) is observed. This poses a 'selection problem'⁴: for given undercooling, the Ivantsov solution admits a continuous family of parabolic solutions, and yet for specified experimental conditions only one is seen. Moreover, it is known that these Ivantsov solutions are 'linearly unstable', meaning that they are unable to maintain their shape during growth⁴.

The first attempts to resolve the stability problem were based on a hope that incorporation of surface tension would involve only a minor modification in shape of the Ivantsov parabolic fronts, while still producing stable solutions below a characteristic length scale. Oldfield¹³ proposed that the selected dendrite was the one moving with the minimum speed (or maximum tip radius), which is stabilized by surface tension. Building on this idea, Langer and Müller-Krumbhaar¹⁴ performed calculations to find this 'marginally stable' solution and further advanced the concept of such a selection principle.

A more complete incorporation of microscopic dynamics has had to await developments in computing and mathematical methods. The surprising result of these studies is that surface tension and surface kinetics are 'singular' perturbations in the dynamical equations for interface evolution, in the sense that

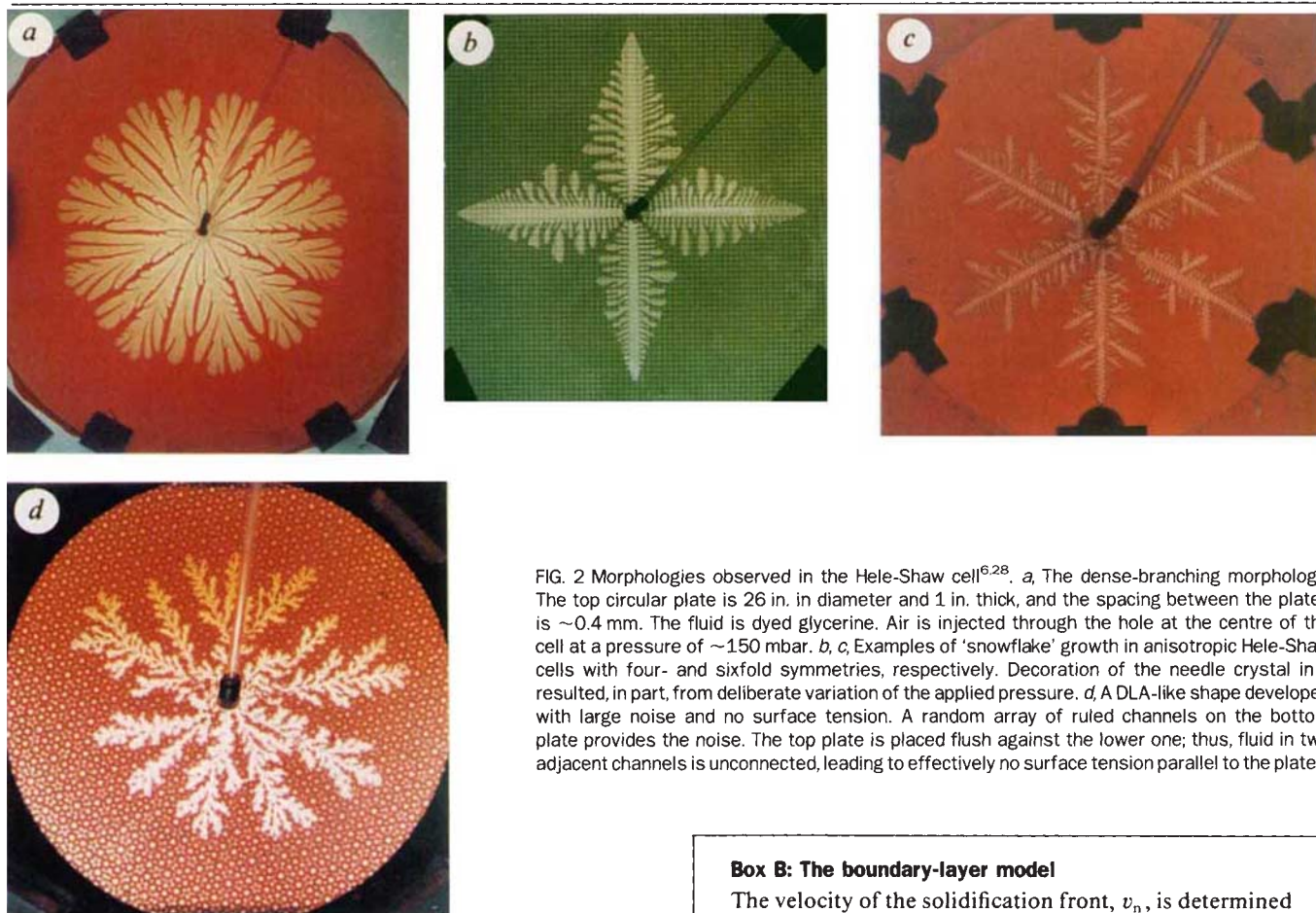


FIG. 2 Morphologies observed in the Hele-Shaw cell^{6,28}. *a*, The dense-branching morphology. The top circular plate is 26 in. in diameter and 1 in. thick, and the spacing between the plates is ~ 0.4 mm. The fluid is dyed glycerine. Air is injected through the hole at the centre of the cell at a pressure of ~ 150 mbar. *b*, *c*, Examples of 'snowflake' growth in anisotropic Hele-Shaw cells with four- and sixfold symmetries, respectively. Decoration of the needle crystal in *c* resulted, in part, from deliberate variation of the applied pressure. *d*, A DLA-like shape developed with large noise and no surface tension. A random array of ruled channels on the bottom plate provides the noise. The top plate is placed flush against the lower one; thus, fluid in two adjacent channels is unconnected, leading to effectively no surface tension parallel to the plates.

they may totally alter the character of the solutions and so must be incorporated from the outset. Thus, the microscopic dynamics cannot be treated as small perturbations of macroscopic solutions; on the contrary, when microscopic dynamics are incorporated from the start, dendritic growth does not occur when surface tension and surface kinetics are isotropic. Instead, tip-splitting fingers develop, leading to the 'dense-branching morphology' discussed below. For dendritic growth, anisotropy is required in the interfacial dynamics¹⁵.

The dense-branching morphology

The Hele-Shaw cell¹⁶⁻¹⁹ provides a simple means of studying pattern formation. It consists of two closely spaced plexiglass plates sandwiching a layer of viscous fluid (we use dyed glycerine). The top plate is circular and open to the air at its edge. Through a tube at the centre of the plate a second, less viscous fluid (air or water) is injected to displace the glycerine.

Figure 2*a* shows an example of the dense-branching morphology (DBM) in the Hele-Shaw cell. It consists of fingers of well defined width confined within a roughly circular envelope. The splitting at the tip distinguishes the fingers from dendrites. This tip-splitting results from the interplay between the macroscopic diffusion field (corresponding to pressure in the Hele-Shaw cell), which tends to make the interface irregular, and the microscopic effects of isotropic surface tension and surface kinetics, which tend to keep the velocity uniform and the interface smooth. The velocity of a point on the interface in the direction normal to the interface is proportional to the pressure gradient at that point. If the less viscous fluid is air, for which the viscosity can be assumed to be zero, the pressure within the injected bubble is constant. In the more viscous fluid (here

Box B: The boundary-layer model

The velocity of the solidification front, v_n , is determined by energy conservation of the latent heat generated at the interface and its diffusion into the melt (ignoring heat diffusion in the solid): $v_n = -(DC_p/L)\nabla_n T$, where D is the thermal diffusion constant⁴. To calculate the velocity and predict the interfacial shape, the temperature throughout the melt must be known at all times. (The dynamics is nonlocal in both space and time because the interface is a free boundary.) For this full solidification (diffusion) problem, the diffusion equation $\partial T/\partial t = D\nabla^2 T$ must be solved with the boundary conditions that far away from the interface $T_\infty = T_m - \Delta$, where Δ is the undercooling and the interface temperature T_i (including microscopic effects) is described in Box A.

The boundary-layer model (BLM) simplifies the diffusion problem by assuming that the entire temperature change occurs within a narrow layer of width l , near the interface. In terms of l , the interface velocity is then $v_n = (DC_p/L)(T_i/l)$. We must now describe the time evolution of l , and this is done using a phenomenological approach. We define the heat content of the boundary layer per unit interfacial length, $H = C_p(T_i - T_\infty)l$. The time evolution of H is determined by heat balance within the boundary layer (along the normal direction) as the interface advances:

$$\frac{dH}{dt} \Big|_n = v_n L - C_p(T_i - T_\infty)v_n - \kappa v_n H + D \frac{\partial}{\partial s} \left(l \frac{\partial T_i}{\partial s} \right) \quad (1)$$

From left to right, the terms represent (1) the production of latent heat; (2) the heat needed to warm the next layer of melt to the surface temperature; (3) the change in heat owing to the increase in arc length, ds , along the interface; and (4) the change in heat resulting from the lateral diffusion of heat along the interface. Each interfacial point moves along the normal to the surface with velocity v_n .

glycerine) the pressure gradient is analogous to the concentration gradient in a very dilute solution at the boundary with a phase of different concentration. The pressure and concentration fields satisfy the Laplace equation²⁰. In many physical cases the Laplace equation may be regarded as the 'infinite diffusion length' limit of the diffusion equation. The diffusion equation itself (see Box B) describes many examples of non-equilibrium growth: for example, solidification from an undercooled metal, precipitation from supersaturated solution or phase separation during amorphous annealing.

A key macroscopic effect of the pressure field at the interface is the diffusive (Mullins-Sekerka) instability²¹, illustrated in Fig. 3b. We consider an interface that is initially circular but develops a bulge. The pressure gradient along the interface is greatest at the tip of the bulge because it is the closest point on the interface to the outer boundary (the local pressure gradient is the pressure drop between the interface and the boundary divided by the distance to the boundary). Because the velocity of a point on the interface is proportional to the local pressure gradient, the bulge grows faster than other parts of the interface. If this instability is the only mechanism acting, then noise or fluctuation in the diffusion field will cause the interface to break up into many growing bulges; these will develop smaller bulges, and so on *ad infinitum*. The result is an invasion of the more viscous phase by the less viscous one without ordering or a characteristic length scale. Experimental realizations of this situation are possible by making the interfacial tension very small, for example by using two miscible fluids²² or by adding glass beads²³. It is also the process that operates during diffusion-limited aggregation (DLA), in which case the diffusive instability leads to a fractal structure^{7-9, 20, 24, 25}.

The microscopic effects of surface tension and surface kinetics compete with the diffusive instability. Surface tension acts to reduce the pressure at the highly curved parts of an interface, and surface kinetics reduce the pressure on the faster-moving portions (see Box A). In Fig. 3c we show the calculated rate of growth or decay of small-amplitude perturbations imposed on the interface, with these effects incorporated (a 'linear stability analysis'^{6, 26, 27}). For simplicity, we consider wave perturbations (Fig. 3b) characterized by the mode number m , the number of waves that fit on the interface. The effects of surface tension and surface kinetics are seen to be too weak to stabilize a circular interface.

Linear stability analysis can be used to investigate the branching rate of the fingers, which may be the best way to characterize the DBM. This branching rate can be studied by finger counting or by Fourier analysis. Figure 3c shows that there is a fastest growing perturbation, with mode number m^* , which produces an interface modulated by m^* finger-like bulges. The value of m^* is determined by the pressure gradient across the system and the surface parameters. As a finger develops, the pressure at the tip is reduced by surface tension and surface kinetics, slowing it down. The finger flattens and eventually splits. It is natural to expect, and has been found experimentally^{6, 26}, that the rate of tip-splitting is such that the number of fingers can be approximated by m^* . Hence m^* embodies the characteristic length scale of the DBM, and reflects the competition between the diffusive instability and microscopic effects²⁸.

The circular envelope of the DBM (observed with circular boundary conditions) is an integral part of the dynamics of the pattern. A naive understanding of how it arises is that if one finger outgrows the others it has more space to spread out; part

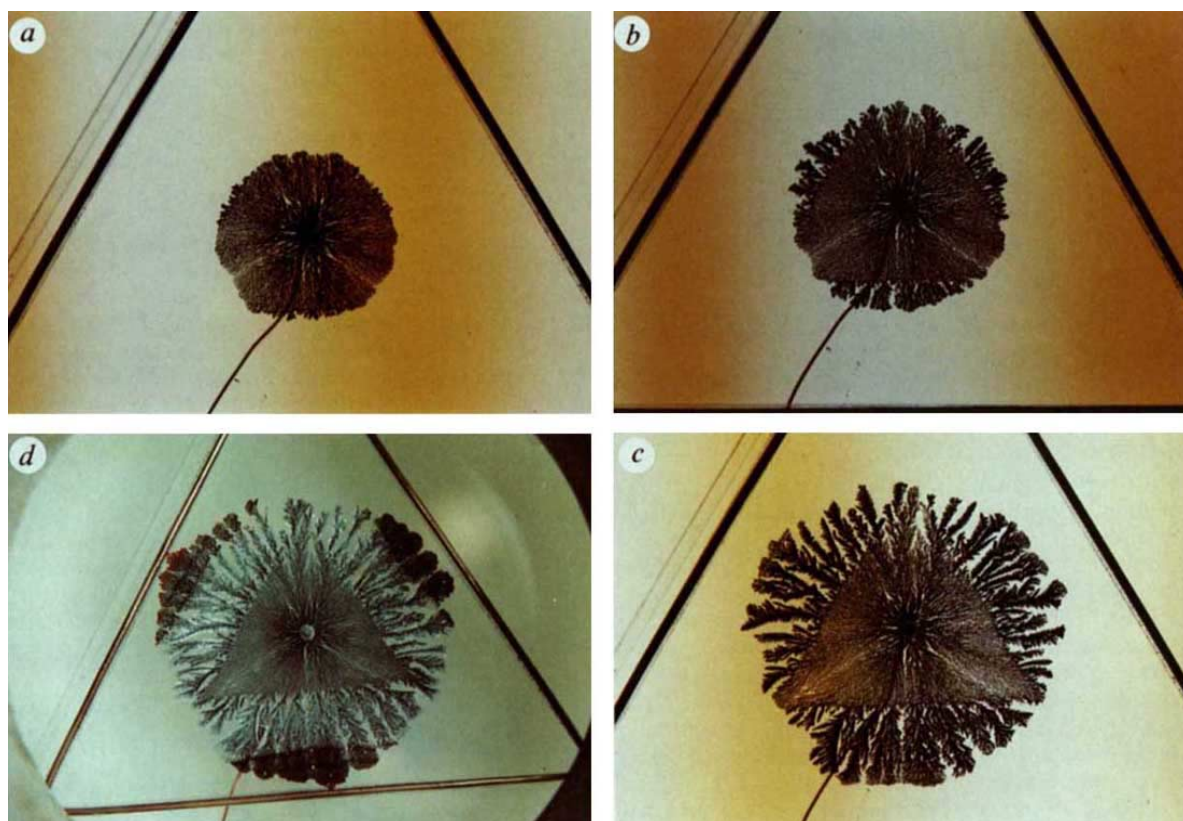


FIG. 6 Morphology transition in electrochemical deposition of zinc from 0.05M ZnSO_4 solution between plexiglass plates spaced about 0.3 mm apart³⁶. The outer anode has a triangular shape (8 cm edge). The first transition ($a \rightarrow b$) is from tip-splitting growth to dendritic growth. The change in colour reflects the change in the microscopic structure of the two

morphologies. The second transition ($c \rightarrow d$) is due to the effect of the copper ions from the outer electrode affecting the microstructure of the deposit. Transitions between two dense-branching morphologies with different branch densities are also observed in electrochemical deposition of copper⁵¹.

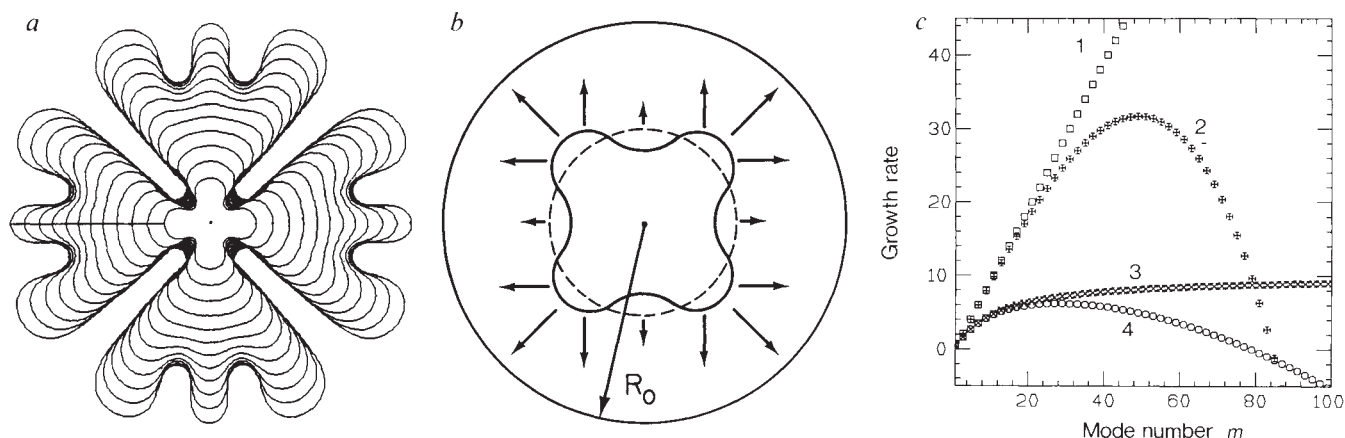


FIG. 3 The DBM and linear stability analysis. *a*, Computer simulation of the time evolution of an isotropic Hele-Shaw cell experiment, showing two cascades of tip-splitting⁵⁰. *b*, Schematic illustration of the Mullins-Sekerka instability²¹. The solid curve is a fourfold deformation ($m=4$) of a circular interface (dashed line). The four bulges are closer to the outer boundary (circle with radius R_0), so the pressure gradient and the velocity (arrows) is larger at their tips. *c*, Results of the linear stability analysis, showing the

initial growth rate as a function of the mode number m for sinusoidal perturbations of a circular interface. Line 1 is for constant pressure along the interface without surface tension or surface kinetics; line 2 is with surface tension included; line 3 is with surface kinetics; and line 4 is with both surface tension and surface kinetics. In the first case there is no fastest-growing mode.

of the flow goes sideways and the finger flattens and slows down. We believe that a nonlinear analysis will be required to understand the growth of this envelope. Recently, a linear stability analysis was proposed to explain the DBM as seen during electrochemical deposition²⁹.

Experiments indicate that, in the absence of pronounced anisotropy, tip-splitting is the generic mode of growth; it is observed, for example, in growth by electrochemical deposition^{30,31}, precipitation from supersaturated solution, solidification from undercooled melts³², amorphous annealing⁶ and spherulitic growth³³. The most pressing unsolved problem is the determination of the branching rates^{34,35} and velocity^{28,33,36} of DBM growth. The latter is especially intriguing because a selection mechanism may operate to give a constant interfacial velocity.

Anisotropy and dendritic growth

The Hele-Shaw cell can also be used to study anisotropic growth, which is analogous to the solidification of a crystalline material. Crystalline anisotropy may be mimicked very simply in the cell by engraving channels on one of the plates³⁷. The fluid velocity is proportional to the square of the plate spacing; because channels modulate the spacing between the plates, the directions along the channels become the preferred growth directions. With an engraved lattice of sixfold symmetry (three sets of parallel channels oriented at 120° to each other), the air bubble adopts shapes reminiscent of snowflakes, with six dendritic arms. (Because snowfall on Mars is composed of CO_2 flakes with fourfold anisotropy, lattices with fourfold symmetry mimic 'Martian snowflakes'.) The formation of dendrites in the

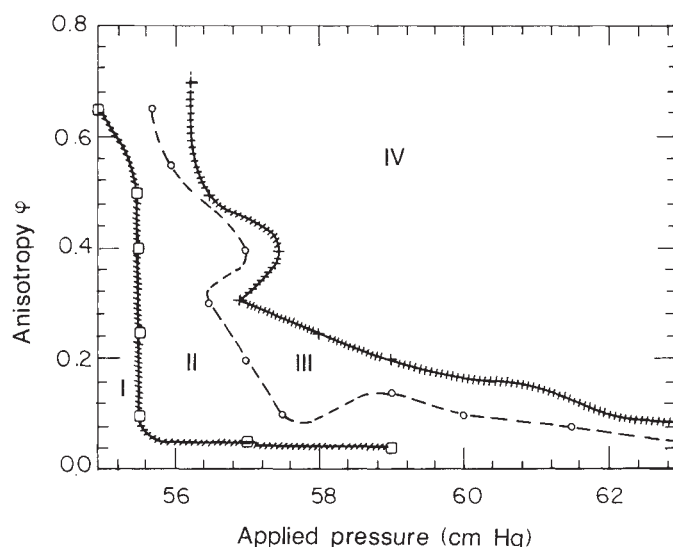
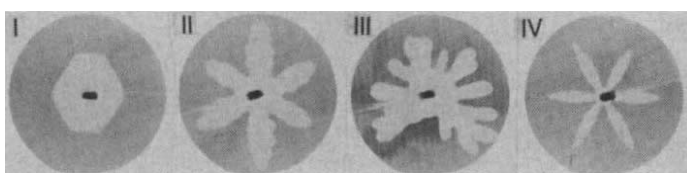


FIG. 4 Morphology diagram for a sixfold anisotropic Hele-Shaw cell^{28,37}. The anisotropy of the cell is quantified by the ratio $\phi = b_1/(b_0 + b_1)$, where b_1 is the depth of the grooves (0.015 in.), and b_0 is the spacing between the top plate and the top of the grooved plate. The morphology regions are: (I) faceted growth; (II) surface-tension dendritic growth (the dendrites are inclined at an angle of 30° to the grooves); (III) tip-splitting growth; and (IV) kinetic dendritic growth (the dendrites grow parallel to the grooves). Cross-hatching on morphology boundaries indicates the possible existence of narrow regions of other morphologies. (For more details see ref. 28). At pressures between regions (II) and (IV), the anisotropies of surface tension and surface kinetics are of comparable strength and cancel each other, leading to vanishing effective anisotropy and thus to tip-splitting growth.

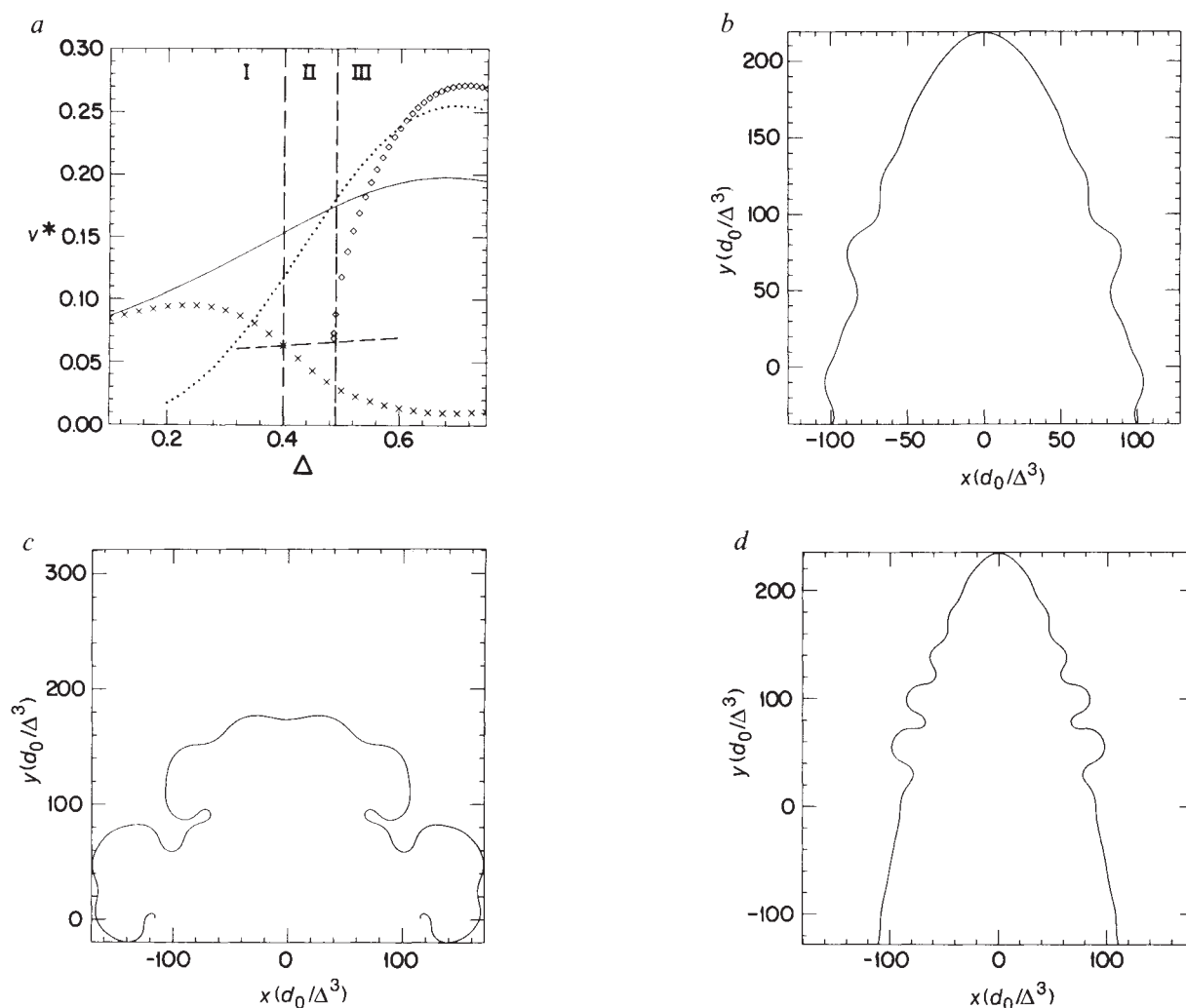


FIG. 5 *a*, The morphology diagram and the needle-crystal selected velocity v^* (dimensionless units) for competing anisotropies in the BLM²⁸. Surface-tension and surface-kinetics anisotropies have preferred growth directions offset by 30°, as in the sixfold Hele-Shaw cell. Crosses indicate the selected velocity of needle crystals pointing in the preferred direction of the surface-tension anisotropy, and diamonds are for crystals pointing in the surface-kinetics preferred direction. The nearly horizontal dashed line represents our calculation of the DBM velocity. The solid line is the selected velocity for the 'surface-tension' needle crystal in the absence of kinetic anisotropy,

and the dotted line is that for the 'kinetic' needle crystal with no surface-tension anisotropy. *b*, Time-dependent simulation of a surface-tension dendrite in regime I; *c*, Tip-splitting growth in regime II; and *d*, surface-kinetic dendrite in regime III. Above a critical undercooling Δ_c , surface-kinetics dendrites have the higher growth velocity and are the observed morphology. There is a discontinuous jump in the velocity at Δ_c , indicating a first-order transition from the DBM to surface-kinetics dendrites. The transition from surface-tension dendrites to the DBM is second order.

Hele-Shaw cell³⁷ provides a graphic demonstration of the role of anisotropy in dendritic growth.

As the applied pressure (the driving force) is varied, the air bubble assumes other shapes. Similarly, as the 'microscopic' conditions are altered, different morphologies emerge. For example, the 'strength' of anisotropy can be changed by varying the spacing between the plates. One can devise a 'morphology diagram' to organize these observations²⁸. Figure 4 depicts a morphology diagram for a cell with sixfold anisotropy. For different values of the applied pressure, one may obtain faceted growth, the DBM or two types of dendrites. As explained below, the existence of two different types of dendrites is particularly significant in an understanding of morphology transitions. Morphology diagrams have also been formulated for electrochemical deposition experiments^{30,31}, Hele-Shaw cells using liquid crystals as the viscous fluid²⁶, and solidification from supersaturated solutions³⁷.

Several questions now arise. When does anisotropy trigger dendritic rather than tip-splitting growth? What is the difference between the dendrites appearing close to equilibrium and those

far from equilibrium? Is there a general principle that allows one to predict the mode of growth for specified conditions from the available morphologies, and thus to explain the morphology diagram?

We can construct an heuristic, albeit far from definitive, argument to understand the selection of tip-splitting or dendritic growth, and the role of anisotropy. Consider a crystal solidifying with a parabolic interface and a finite surface tension. The tip is the coldest point on the interface (see Box A). Thus there is a heat gradient along the interface, and heat flows towards the tip. This warming slows the advance of the interface, while the corresponding cooling of points at the sides of the tip causes these points to advance faster and to overtake the tip. Such interfacial heat-balance dynamics probably accounts for tip-splitting growth. For dendrites to form, heat flow towards the tip must be suppressed; this happens in the presence of crystal-line anisotropy. For large enough anisotropy (of the order of one part in a hundred), the tip is no longer the coldest point along the interface, and a subtle interplay may develop between the anisotropy and the shape selection of the needle crystal. For

Box C: Microscopic solvability criterion

We seek steady-state (constant-velocity and shape-preserving) solutions of the BLM with a needle-crystal-like shape^{39,40}. If an interface advancing in the $\theta = 0$ (ordinate) direction with constant velocity v_0 preserves its shape, then the interface velocity in the normal direction is $v_n = v_0 \cos \theta$, and in the tangential direction is $v_t = v_0 \sin \theta$. The properties of the boundary layer are stationary in the moving frame: in other words, the time derivative of H at fixed arclength vanishes. That is,

$$\left. \frac{dH}{dt} \right|_s \equiv \left. \frac{dH}{dt} \right|_n - \left(\frac{\partial H}{\partial s} \right) v_t = 0 \quad (2)$$

and the derivative along the normal direction is equation (1) (Box B). In the absence of surface tension and surface kinetics, the interfacial temperature is constant, $T_i = T_m$, and equation (1) can be solved exactly. For a given value of undercooling there exists a continuous family of parabolic Ivantsov-like solutions. The singular nature of surface effects manifests itself in the fact that these solutions are destroyed when either surface tension or surface kinetics are included. These singular perturbations change completely the nature of the solution and lead to tip-splitting rather than needle-crystal growth. Anisotropy is also a singular perturbation. The surprising discovery is that when it is included needle-crystal-like solutions are recovered.

To demonstrate the existence of such solutions, equation (2) is rewritten as three first-order coupled nonlinear differential equations⁴⁰. These equations describe a 'flow' in the three-dimensional space whose coordinates are θ , T_i and $\lambda = dT_i/ds$. There are two special fixed points: $(d\theta/ds = dT_i/ds = d\lambda/ds = 0)$ at $\theta = \pm \pi/2$, $T_i = T_m$ and $\lambda = 0$. A needle-crystal solution is a trajectory connecting these two fixed points. The problem is posed in terms of $\lambda(s=0)$, the 'mismatch function'. Because the temperature must be symmetric about the origin ($s=0$, $\theta=0$), the mismatch function must vanish for an eigenvalue v_0 if a needle crystal is to exist. In the absence of anisotropy there is no selected needle crystal. But for any finite level of anisotropy there is a discrete set of eigenvalues, $\{v_0\}$, for which the mismatch function vanishes. The phrase 'microscopic solvability' reflects the subtle interplay, in the determination of these eigenvalues, between the macroscopic driving force of the undercooling and microscopic interfacial effects.

a given anisotropy, only a needle crystal with a particular tip velocity and curvature will have its coldest point at the appropriate temperature and distance from the tip to exactly balance the tip-splitting tendency. This is the origin of the 'solvability' criterion (Box C). It is found that, instead of the original (Ivantsov) continuous family of parabolas, only a discrete set of (nearly parabolic) needle-crystal solutions can satisfy this criterion. A stability argument is required to select one solution from this discrete set. If a needle crystal is perturbed, say by introducing a bulge near the tip, the perturbation will grow as a result of the diffusive instability. This bulge grows outwards at a fixed position in space, so as the tip advances the bulge moves backwards relative to the tip. Only for the fastest-growing needle crystal does the bulge move backwards faster than its growth rate, allowing the tip to regain its original shape. Thus, only the fastest growing needle crystal can survive the effect of the diffusive instability.

This microscopic solvability criterion was discovered independently using the geometrical³⁹ and boundary-layer⁴⁰ models. It has since been shown that the same mechanism is present in

the full solidification problem, and analytical methods have been developed to calculate the selected velocity in the limit of small undercooling and small anisotropy⁴¹⁻⁴³. There is good agreement with time-dependent supercomputer simulations of the full solidification problem⁴⁴; the selection hypothesis is, however, yet to be tested comprehensively against experiment.

A complete theory of dendritic growth must also explain the dynamic development of side branches. Much attention is being given to this question⁴⁵⁻⁴⁸; the debate focuses on the relative roles of noise and deterministic dynamics. The question is whether side branches grow as a result of noise that triggers a diffusive instability (such that a linear stability analysis would be sufficient to predict their evolution) or whether, as we believe, an additional solvability principle is required.

Selection hypothesis for morphology transitions

With the discovery of the microscopic solvability criterion outlined in Box C, the problem of dendritic growth seemed to be solved. But the anisotropic Hele-Shaw experiment and the boundary-layer model (BLM) (see Box C) expose a problem: dendrites are not always observed when anisotropy is present. As the driving force (pressure in the Hele-Shaw cell and undercooling in the BLM) is decreased, tip-splitting occurs below a critical value. (Similar behaviour is observed experimentally in the solidification of water³².) The selection principle for dendritic growth, however, predicts that whenever anisotropy is present, a specific dendrite (corresponding to the fastest-growing needle crystal) exists and is linearly stable. The observation of tip-splitting under growth conditions appropriate to dendrites raises the possibility that morphologies can coexist. As only one morphology is selected experimentally, microscopic solvability must be incomplete—a more general principle is needed.

A plausible extension of the solvability criterion is the more general principle that the dynamically selected morphology is the fastest-growing one²⁸. That is, if more than one morphology is possible, only the fastest one is nonlinearly stable and hence observable. Thus, we infer that below a critical driving force the velocity of the DBM is greater than the velocity of the dendritic solution predicted for the same parameters, and so the former is selected. To test this hypothesis, we have studied the effect of competing anisotropies using the BLM. We included anisotropies in both surface tension and surface kinetics, with preferred growth directions offset by 30°. The results for the selected velocity along both the surface-tension and surface-kinetics directions (Fig. 5) show that above a critical undercooling both types of dendrites are possible, but those with highest velocity are controlled by the surface kinetics. Time-dependent simulations of the BLM demonstrate that the 'surface-kinetics' dendrites do indeed comprise the dynamically selected morphology in this regime. Further studies of experimental systems and comparison with theoretical models are required to test this 'fastest-growing' selection hypothesis.

An analogy can be drawn between phase diagrams and morphology diagrams. At equilibrium, the phase that minimizes the free energy, for a given set of state variables, is the selected one, irrespective of the prior history of the system. By contrast, non-equilibrium growth processes are time dependent, so it is not clear *a priori* that a morphology diagram can exist. The question is whether appropriate control parameters describing the growth conditions, analogous to the state variables of thermodynamics, can be identified such that the morphology depends only on these parameters and not on initial conditions. One finds that morphology diagrams can indeed be mapped out for experimental systems, and are reproducible as a function of the control parameters. It follows that a corresponding selection principle, which would permit theoretical prediction of growth morphologies, must exist.

The analogy between equilibrium phase diagrams and morphology diagrams can be carried further. Two types of morphology transition can be identified²⁸: the first shows a

discontinuous jump in the velocity at the transition point (and is hence classified as a first-order morphology transition), whereas for the other (characterized as second-order), the velocity itself is continuous as the morphology changes but there is a discontinuity in its derivative. Such morphology transitions are found both experimentally and theoretically. In a study of solidification from supersaturated NH_4Cl solutions, Chan *et al.*³⁷ found that there was either a jump discontinuity (first order) or a discontinuity in the slope (second order) of the observed dendritic velocity as a function of supersaturation, corresponding to changes in crystallographic orientation of the growing dendrites. First- and second-order morphology transitions in the BLM are shown in Fig. 5.

Experiments on growth by electrochemical deposition produce results that are in qualitative agreement with this characterization of morphology transitions. Sawada *et al.*³⁰ have plotted the interfacial velocity against applied voltage and found sudden changes in slope when the morphology changes. We have observed discontinuities in both slope and magnitude of the interfacial velocity, depending on the growth parameters. The morphology transition in electrochemical deposition shown in Fig. 6 demonstrates two key aspects of such transitions: the transition is sharp and is accompanied by a change in the microstructure of the growing deposit (in this case coincident with a colour change). These observations lend support to the concept of morphology transitions.

Concluding remarks

The existence of a morphology diagram indicates that a selection principle must exist. Is this principle the 'fastest-growing morphology' hypothesis that we have proposed? We believe that this may not be the most general principle but is at least a step in the right direction.

When a system is driven out of equilibrium by the imposition of a gradient in one of the thermodynamic variables (such as the temperature or the concentration), the response of the system is described by the conjugate flux (the heat flux and particle flux, respectively). These fluxes may in general be viewed as the rate of entropy production, or the rate of approach towards global equilibrium. In growth processes specifically, the driving force (for example, the undercooling in solidification) is the equivalent of the thermodynamic gradient. The average velocity measures the rate of approach towards equilibrium, and therefore represents a response function. But the global rate of change of the free energy (at the interface) is given by the integral of the velocity along the interface. Thus, by 'average velocity' we mean the velocity weighted according to the geometry of the interface, and thus we take into account the global shape of the object. We speculate that the average velocity is an important variable but is by no means the only one. It should have a conjugate variable (at present unknown) that will be characteristic of the equilibrium properties of the interface and the selected growth morphology.

The 'fastest-growing' selection principle is probably a good approximation to the general selection principle when the system is far from equilibrium, in which case the growth rate is the dominant factor in the competition between morphologies. An analogy can be drawn with the high-temperature limit of an equilibrium system coupled to a heat bath, where entropy is dominant: thus, we expect that far from equilibrium, entropy production is dominant in morphology selection. □

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Type I macrophage scavenger receptor contains α -helical and collagen-like coiled coils

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The macrophage scavenger receptor is a trimeric membrane glycoprotein with unusual ligand-binding properties which has been implicated in the development of atherosclerosis. The trimeric structure of the bovine type I scavenger receptor, deduced by complementary DNA cloning, contains three extracellular C-terminal cysteine-rich domains connected to the transmembrane domain by a long fibrous stalk. This stalk structure, composed of an α -helical coiled coil and a collagen-like triple helix, has not previously been observed in an integral membrane protein.

IN atherosclerosis, the endocytosis of plasma lipoprotein cholesterol by monocyte-macrophages results in the formation of foam cells¹⁻³. *In vitro* and genetic studies indicate that the well-known pathway involving the low-density lipoprotein (LDL) receptor is not required, and may not even be involved⁴. The identification of a macrophage receptor^{5,6} mediating the endocytosis of chemically modified LDL raised the possibility that this AcLDL (acetylated LDL) or scavenger receptor plays a key part in atherogenesis^{3,6,7}. Certainly the foam cells rich in cholesteryl ester formed when cultured macrophages are exposed to appropriately modified LDL are strikingly similar to foam cells in atherosclerotic plaques⁶. Recent *in vivo* studies using an animal model of familial hypercholesterolaemia^{3,8-10} support the view that modified LDL and the scavenger receptor are critically involved in the development of atherosclerosis.

The modifications of LDL that convert it into a ligand for the scavenger receptor^{3,5,7} include acetylation and oxidation. A surprisingly diverse group of macromolecules, including maleylated bovine serum albumin (M-BSA), polyvinyl sulphate (PVS), and polyinosine (poly(I)), can inhibit the binding of modified LDL to the scavenger receptor and its subsequent endocytosis⁶.

We have previously reported the isolation of a trimeric bovine scavenger receptor of relative molecular mass 220,000 (M_r 220K) composed of glycoprotein subunits of M_r 77K (ref. 11; see also refs 12 and 13). We have now deduced the primary structure of this receptor by first determining its partial amino-acid sequence and by using that as a starting point for the cloning and expression in COS cells of the corresponding complementary DNA. We have isolated two closely related cDNAs, designating their products scavenger receptors type I and type II (ref. 14).

The deduced structures of the two receptors, both integral membrane proteins, are remarkable in that they each contain a collagenous domain. There are six consecutive domains in the type I receptor, as follows: (I) N-terminal cytoplasmic domain; (II) transmembrane domain; (III) spacer domain; (IV) α -helical coiled-coil domain; (V) collagenous domain; and (VI) C-

terminal cysteine-rich domain. Domain VI is not present in the type II receptor and is not required for the binding of AcLDL¹⁴. These cloned genes should be useful for investigating the structure and physiological and pathophysiological functions of this novel class of differentially expressed¹⁵ cell-surface receptor.

Amino-acid sequencing and cDNA cloning

We determined the sequences of two peptides, I and II, isolated after digestion of the receptor by cyanogen bromide (Fig. 1). An anti-peptide I antibody recognized a protein (Fig. 1b, right lanes) that had the same electrophoretic mobility as the highly purified receptor under nonreducing (trimer and dimer) and reducing (monomer) conditions (silver staining, left lanes). We used oligonucleotide probes containing 5-fluorodeoxyuridine (5-FU) to clone corresponding cDNAs from bovine lung libraries (Fig. 4 legend). We substituted 5-FU at positions of cytosine-thymidine to reduce probe degeneracy¹⁶.

Verification of cDNA clone identity

Tissue and cell-type expression. Northern-blot analysis showed relatively high levels of expression of the cloned gene in bovine alveolar macrophages and bovine lung (2.2-kilobase (kb) band, Fig. 2a), but not in brain and muscle. Tissue-specific RNA expression corresponded to tissue-specific expression of the receptor protein (immunoblots in b). We also detected weak hybridization to a 1.5-kb band in alveolar macrophage, lung and muscle. The significance of this is uncertain. In the human monocytic leukaemia cell line THP-1, expression of the scavenger receptor is dramatically increased after phorbol ester treatment^{11,17}, and we detected RNA expression only after treatment with phorbol ester (Fig. 2a, 4.4- and 5.5-kb bands). The expression of the cloned gene therefore exhibits the tissue-type and cell-type specificity of the scavenger receptor^{6,11,15,17}, and the bovine and human receptors share similar sequences (confirmed by Southern-blot analysis; data not shown). The differences in the sizes of the human and bovine messenger RNAs could be due to differences in the lengths of the untranslated portions of the messages.

Complementary DNA expression in COS cells. We generated a scavenger receptor expression vector (plasmid pXSR7) and transfected it into COS M6 cells (Fig. 3). Plasmid pXSR7 conferred high-affinity and saturable receptor activity (Michaelis constant $K_m \sim 1 \mu\text{g}$ AcLDL protein per ml, or 2 nM AcLDL) with the same specificity as the macrophage scavenger receptor⁶. For example, AcLDL, maleyl BSA, poly(I) (Fig. 3b), and polyvinyl sulphate (data not shown), inhibited the internalization and degradation of ¹²⁵I-labelled AcLDL, whereas LDL, BSA, and poly(C) did not. We have therefore cloned a scavenger receptor.

Primary structure analysis

The insert in clone plasmid pBSR7 includes a 1,359-base pair (bp) open reading frame encoding a protein of 453 amino acids with a calculated M_r of 49,975 (Fig. 4). Key structural features are illustrated in Figs 4, 5 and 6. The predicted protein sequence defines six domains: (I) N-terminal cytoplasmic (amino-acid

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residues 1–50); (II) transmembrane (51–76); (III) spacer (77–108); (IV) α -helical coiled-coil (109–271); (V) collagenous (272–343); and (VI) C-terminal cysteine-rich (344–453). The receptor contains eight cysteines, one each in domains I and III, and six in domain VI.

Domain I: cytoplasmic N terminus. The receptor contains a single hydrophobic stretch of 26 amino acids (domain II, Fig. 4) but does not have a typical N-terminal signal peptide sequence¹⁸. The polarity of the amino acids immediately next to this hydrophobic domain (positive facing the cytosol)¹⁹ and the location of all seven potential N-linked sugar attachment sites (domains III and IV) strongly indicate that the receptor is an integral membrane protein with a single membrane-spanning domain and an N-terminal cytoplasmic domain. Many of the potential N-linked sites are probably glycosylated, because N-linked sugars account for as much as 15–20K of the 77K apparent M_r of the monomer subunits¹¹. Signals were not observed at the two relevant asparagine locations during sequencing of peptides

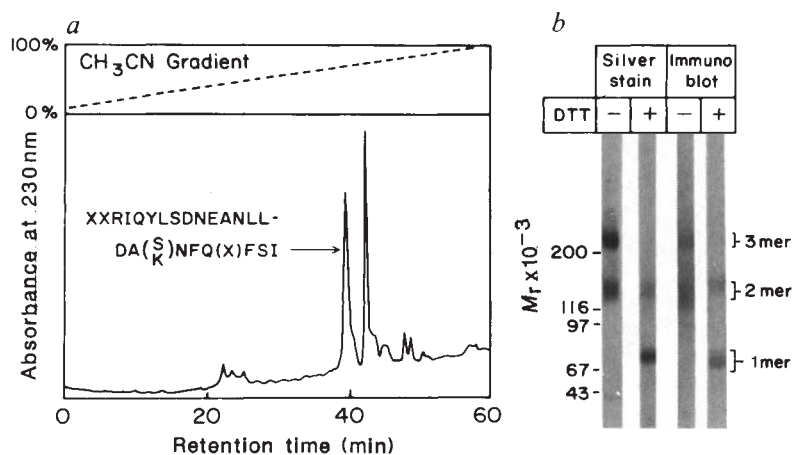
I and II, indicating that these sites are glycosylated. Also, the predicted collagenous structure of domain V (see below) has only been previously observed in secreted proteins (for example, complement C1q (ref. 21)). There are two potential protein kinase substrate sites (Arg-X-X-Ser/Thr; ref. 22) of unknown function in the N-terminal domain, Arg-Ser-Val-Thr (residues 26–29) and Arg-Met-Lys-Ser (residues 45–48).

Two classes of coiled coils

Domain IV: α -helical coiled coil. A short proline-containing spacer (domain III) connects the transmembrane domain with extracellular domain IV. Domain IV contains as many as 23 seven-amino-acid 'heptad' repeats. From the sequences of these repeats we strongly predict an α -helical coiled-coil structure (presumably three-stranded) held together by an interhelical hydrophobic core of aliphatic residues at positions a and d of the heptad (see Fig. 5)²³. The heptad sequences from Val 154 to Leu 228 are especially good candidates for the coiled-coil struc-

FIG. 1 Isolation, sequencing and characterization of a peptide obtained from the bovine scavenger receptor. *a*, Reverse-phase HPLC of peptides derived from CNBr-digested receptor. *b*, Silver staining of highly purified receptor (left) and anti-peptide antibody immunoblotting of crude bovine lung membrane proteins (right).

METHODS. Scavenger receptor (immunoaffinity purified from four bovine lungs¹¹) was precipitated with chloroform-methanol³⁸, solubilized in 1.5 ml guanidine thiocyanate (2M), 10 mM Tris-HCl buffer, pH 8.0, and subjected to HPLC (RP300 column, gradient of acetonitrile in 0.1% trifluoroacetic acid). The receptor was solubilized in 100 μ l formic acid (70%), and digested with CNBr overnight. The digestion products were rechromatographed using the same procedure (*a*). Peptide I (~100 pmol; arrow) was sequenced using an Applied Biosystems model 470 gas-phase sequencer. The sequence was (X)(X)RIQYLSNEANLLDA(S/K)NFQ(X)FSI (single-letter amino-acid code; (S/K) indicates ambiguity at this position). Independently, the sequences of CNBr peptides I and II ((X)LLN(X)(X)NDLRL(X)D) isolated by electroblotting³⁹ were determined. A synthetic peptide (1 mg; RIQYLSNEANLLDASNFQ) was coupled using 1-ethyl-3-(3-dimethyl-7-aminopropyl)-carbodiimide HCl (ref. 40) to porcine thyroglobulin (5 mg) and used (four injections) to generate a rabbit anti-peptide antibody. Nonreduced or reduced (10 mM DTT) specimens of either immunoaffinity and RP300 chromatographically purified bovine scavenger receptor¹¹ (*b*, left lanes) or crude lung membrane proteins (partially purified



using hydroxylapatite chromatography¹¹, right lanes) were subjected to SDS-PAGE. Alkylating agents were not used to prevent disulphide interchange during preparation. The purified receptor was visualized by silver staining (left lanes). Receptor in the membrane proteins (right lanes) was visualized by immunoblotting with protein A-purified anti-peptide antibody (1:1,000 dilution) using goat anti-rabbit IgG conjugated with alkaline phosphatase (1:2,000 dilution; Stratagene)¹¹.

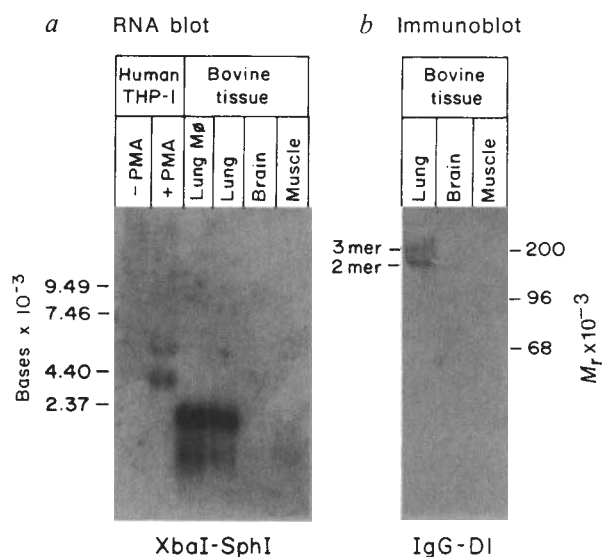


FIG. 2 Tissue-type and cell-type specific expression of scavenger receptor messenger RNA. *a*, RNA blot hybridization. Poly(A)⁺ RNAs were obtained from a human monocytic leukaemia cell line, THP-1, before and after differentiation into macrophage-like cells by phorbol ester treatment, and from bovine alveolar macrophages (M ϕ), and bovine lung, brain and muscle tissue. A fragment of plasmid pBSR7 was radiolabelled and used as the probe. *b*, Immunoblot analysis of the tissue distribution of bovine scavenger receptors. Membrane proteins partially purified from the indicated bovine organs were immunoblotted using anti-bovine scavenger receptor monoclonal antibody IgG-D1.

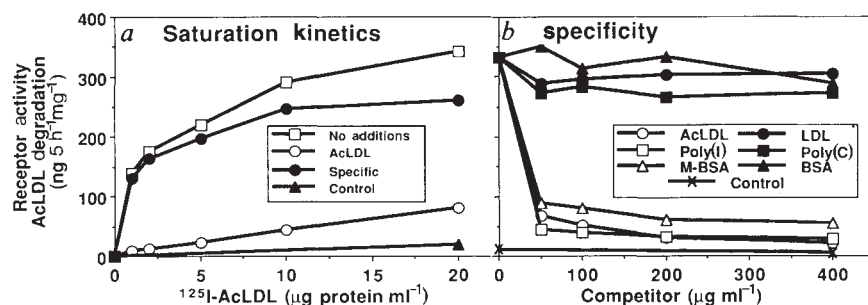
METHODS. *a*, THP-1 cells were cultivated with or without 200 nM phorbol 12-myristate 13-acetate (PMA) for three days and bovine alveolar macrophages were prepared as previously described¹¹. Poly(A)⁺ RNAs (3 μ g) from these cells and from bovine organs⁴¹ were fractionated by electrophoresis, and blotted onto a cellulose nitrate membrane. The *Xba*I-*Sph*I fragment of plasmid pBSR7 (354 bases, nucleotides 682–1,035 in Fig. 4) was radiolabelled with ³²P, and incubated with the membrane at 42 °C in the presence of 40% formamide. The membrane was washed with 2 $\times$ SSC containing 0.1% SDS at 55 °C. *b*, Freshly isolated bovine lung, brain and muscle (5 g of each) were partially purified by maleyl-BSA ligand-affinity chromatography¹¹ and immunoblotted (1.25 g equivalent of tissue per lane) using the monoclonal anti-bovine scavenger receptor antibody IgG-D1 (ref. 11).

ture. Indeed, the sequence even includes a 'skip' residue (Asn 203), as is present in myosin^{23,24}. Such skips could alter the superhelical pitch or represent hinges that change the direction of the superhelical axis. It is striking that the charge density increases significantly beyond the skip (Fig. 5, right), indicating that the skip could demarcate a structurally and perhaps functionally distinct subdomain. All five potential *N*-linked sugar sites in domain IV occur at positions constrained to be hydro-

philic by the amphipathic coiled-coil model. The coiled-coil structure could play an important part in the assembly of the functional trimeric receptor¹¹. The presence of histidines at a/d-type positions 168 and 260 raises the possibility that the stability of the structure exhibits pH dependence at physiologically relevant pHs. This pH dependence could be involved with ligand release in acidic intracellular compartments^{25,26}.

FIG. 3 Transient expression of (a) high affinity and (b) specific scavenger receptor activity in plasmid pXSR7-transfected COS cells.

METHODS. The type I scavenger receptor expression vector plasmid pXSR7 was constructed from plasmid pBSR7 (see legend to Fig. 4 for cloning details) by introducing the coding region of the receptor cDNA into the expression vector plasmid pCDNA1 (Invitrogen), a modified version of plasmid pCDM8 (ref. 42). Reagents were from commercial sources or were prepared as previously described^{5,6}. On the first day, COS M6 cells (gift from D. Housman) were set at 1.5×10^6 cells per 100-mm dish in DMEM containing 10% fetal bovine serum, penicillin and streptomycin (medium A). On day 1, the cells were transfected with plasmid pXSR7 or the control plasmid pCDNA1 ($4 \mu\text{g ml}^{-1}$) following the method of Seed⁴². On day 2, the transfected cells were harvested with trypsin and reset into six-well dishes at a concentration of 1×10^6 or 0.8×10^6 per well for assays performed on day 4 or 5, respectively. *a*, The indicated amounts of ^{125}I -labelled AcLDL in the absence (duplicate determinations, $\square$) or presence (single determinations, $\circ$) of $400 \mu\text{g protein ml}^{-1}$ unlabelled AcLDL were added to the monolayers of cells transfected with



plasmid pXSR7, and receptor activity measured as the degradation of ^{125}I -labelled AcLDL as previously described⁵. Specific degradation was calculated by subtraction ($\bullet$). Control values for the specific degradation of ^{125}I -labelled AcLDL by cells transfected with plasmid pCDNA1 were also determined ($\blacktriangle$). *b*, In a separate experiment, the degradation of $5 \mu\text{g protein ml}^{-1}$ ^{125}I -labelled AcLDL in the absence (triplicate determinations) or presence (duplicate determinations) of the indicated amounts of competitors was measured for cells transfected with plasmid pXSR7 or control plasmid pCDNA1 ($\star$, AcLDL used as the competitor).

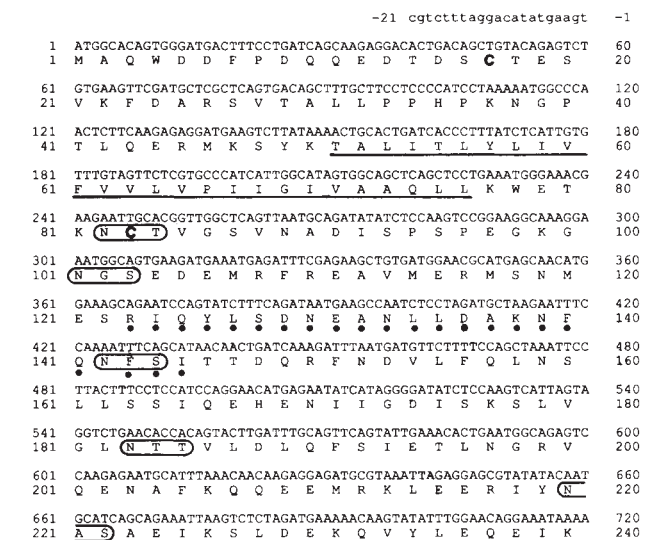


FIG. 4 Nucleotide sequence of the bovine scavenger receptor from plasmid pBSR7 (top) and alignment of the deduced amino-acid sequence (bottom; single-letter code). The sequence surrounding the first ATG (bases 1–3) is consistent with consensus initiation sequences⁴³ and this codon is in frame with those encoding peptides I and II. The putative transmembrane domain is underlined, the amino acids identified by protein sequencing are indicated by underlying filled circles, potential *N*-linked glycosylation sites are enclosed by boxes with rounded corners and collagen-like Gly-X-Y repeats are indicated by boxes with square corners. Cysteine residues are in bold. Plasmid pBSR7 contains 21 and 212 base pairs of 5'- and 3'-untranslated sequence, respectively, but does not contain a polyadenylation signal or a poly(A) tail. Sequencing of a clone isolated from an oligo(dT)-primed library confirmed the sequence surrounding the stop codon.

METHODS. A size-fractionated (>800 base pairs) randomly primed bovine lung poly(A)⁺ (ref. 41) cDNA library in λ ZAPII (Stratagene) was constructed,

amplified, and 5×10^5 plaques were screened (hybridization at 37°C , washing at 50°C in $6 \times \text{SSC}$ with 0.1% SDS) with a pool of ^{32}P end-labelled 41-mer oligonucleotides (5'-ATFCAGTAFFT(F/G)TC(F/G)GAFAAFGAGGC(F/G)AAFFT-(F/G)FT(F/G)GAFGC-3'; F, 5-fluorodeoxyuridine) based on the sequence RIQYLNDSNEANLLDA from peptide I. Positive clones were purified and excised *in vivo* to form pBluescript plasmids. The inserts were screened by blot hybridization using four pools of 17mer oligonucleotides (A(A/G)-(A/G)TTNGC(C/T)TC(A/G)TT(A/G)TC, where N is a different nucleotide in each) and two pools of 26mer oligonucleotides (pool III, AT(F/A)CA(G/A)TAFF-TTCGGAFAAFGA(G/A)GC; pool IV, AT(F/A)CA(G/A)TAFFTIAGFGAFAFGA-(G/A)GC). Five positive clones (including pBSR7 and pBSR3¹⁴) were sequenced by the dideoxy-chain-termination method—all contained sequences encoding peptides I and II. Additional overlapping clones were isolated from an oligo (dT)-primed bovine lung cDNA library (data not shown).

Domain V: collagen triple helix. Domain V is the only portion of the receptor with significant similarity to regions of other proteins. We can infer from its 24 uninterrupted Gly-X-Y tripeptide repeats that it forms a collagenous triple helix²⁷. In collagens, residue Y is often either proline or lysine, which are frequently hydroxylated post-translationally to stabilize the trimeric structure. In domain V, 14 out of the 24 Y residues are proline or lysine. In addition to extracellular matrix collagens, other proteins have collagenous triple helical domains (for example, complement C1q (ref. 21), pulmonary surfactant apoprotein²⁸, asymmetric acetylcholine esterase²⁹ and serum mannose-binding protein³⁰). Sequence identities between domain V and other molecules that contain Gly-X-Y repeats in the National Biomedical Research Foundation data base were not particularly high (for example, 51.4% identity over 74 residues of human type IV $\alpha 1$), considering that one-third of these residues are glycine, and many are proline or lysine.

Domain VI has many glycines (14.5%) and contains six cysteines that could be involved in intrachain or interchain disulphide bonds, or both. Each of the four C-terminal cysteines are separated by nine residues containing three to four charged side chains.

Discussion

The bovine macrophage scavenger receptor is a trimeric membrane protein of $M_r \approx 220K$, composed of 77K glycoprotein subunits, 15–20K of which is due to *N*-glycosylation¹¹. Complementary DNA clones encoding two different forms of the receptor, type I and type II (ref. 14) have been isolated and characterized. The calculated M_r of the type I receptor subunit is 49,975. It has seven potential *N*-glycosylation sites and contains six distinct domains (Figs 4–6). Post-translational hydroxylation of proline and lysine residues in the collagenous

domain and subsequent glycosylation of some hydroxylysines could occur²⁶ and contribute to the apparent size differences seen on electrophoresis (diffuse receptor bands, Fig. 2; and ref. 11).

We can predict from the primary structure of the receptor that it assembles into a trimer. Domains IV and V represent 52% of the protein, and from their sequences we can predict folding into two distinct trimeric quaternary structures. Domain IV consists of a heptad-based α -helical coiled coil, similar to that in the trimeric haemagglutinin of the influenza virus²⁰. The heptad repeat in the scavenger receptor (163 amino acids) is three times longer than the 52-residue (76 Å) coiled coil in haemagglutinin. Immediately next to the coiled coil in the scavenger receptor is a stretch of 24 Gly-X-Y triplets which probably fold into a collagen-type triple helix²⁶. Trimeric scavenger receptors could aggregate into higher-order oligomers, as is the case for complement C1q (ref. 21), pulmonary surfactant apoprotein³¹, and conglutinin³². If the scavenger receptor is composed of higher-order oligomers, its structure could differ from that shown in Fig. 6. For example, it could be similar to hexameric C1q (a trimer of dimers); it could have two trimeric collagen-like domain Vs contiguous with three dimeric coiled-coil domain IVs. Although secondary and quaternary structure predictions from primary sequence data should always be interpreted with caution and the disulphide bonding pattern and subunit stoichiometry remain to be defined precisely, it seems likely that about 60% of the extracellular domain of the type I receptor consists of a long fibrous stalk (domains IV and V) on which there are three globular C-terminal cysteine-rich domains. This is consistent with the previous observation of ligand binding to the trimeric, but not the dimeric or monomeric, form of the purified scavenger receptor¹¹. The purified ligand-binding trimeric form of the scavenger receptor¹¹ could be either a

FIG. 5 Heptad repeats and helical net diagram of scavenger receptor domain IV. *a*, Heptad repeats in domain IV with potential *N*-linked glycosylation sites underlined. Many fibrous proteins are constructed from parallel right-handed amphipathic α -helices (3.5 residues per turn) which coil around each other to form a left-handed supercoiled fibre²³. The interhelical contacts are often mediated by extensive hydrophobic interactions on the regularly repeating faces of the helices. The common repeat is a seven-amino acid heptad (a, b, c, d, e, f and g), which is usually characterized by aliphatic hydrophobic side chains (leucine, isoleucine, valine) at positions a and d and charged and polar side chains at the other positions. Lysine and arginine are occasionally found in position a, but rarely in position d, and aspartic acid and glutamic acid are usually excluded from position a. *b*, An α -helix (3.5-residue pitch, amino acids 98–272) was projected onto a cylinder and unrolled to indicate the proposed spatial relationships of the side chains. In *b* (left), residues in positions a and d of the coiled-coil heptad repeats are enclosed by circles (hydrophobic and basic residues) or diamonds (polar residues). A skip at residue Asn 203 shifts the pattern across the helix surface so that the residues in the a/d positions are located at e/a^{23,24}. In *b* (right), the positions of hydrophobic side chains are indicated by open circles and charged residues by filled circles containing plus or minus signs to indicate the charge. Neutral hydrophilic residues are marked by small dots. An increase in the charge density is seen after the skip.

Residue no.	Heptad Position						
	a	b	c	d	e	f	g
267–271	I	T	L	L	Q		
260–266	H	S	Q	T	L	K	N
253–259	L	R	L	K	D	W	E
246–252	L	N	N	I	T	N	D
239–245	I	K	G	E	M	K	L
232–238	Q	V	Y	L	E	Q	E
225–231	I	K	S	L	D	E	K
218–224	I	Y	N	A	S	A	E
211–217	M	R	K	L	E	E	R
204–210	A	F	K	Q	Q	E	E
200–203	V	Q	E	N	<-skip		
193–199	I	E	T	L	N	G	R
186–192	V	L	D	L	Q	F	S
179–185	L	V	G	L	N	T	T
172–178	I	G	D	I	S	K	S
165–171	I	Q	E	H	E	N	I
158–164	L	N	S	L	L	S	S
151–157	F	N	D	V	L	F	Q
144–150	S	I	T	T	D	Q	R
137–143	A	K	N	F	Q	N	F
130–136	N	E	A	N	L	L	D
123–129	R	I	Q	Y	L	S	D
116–122	R	M	S	N	M	E	S
109–115	F	R	E	A	V	M	E

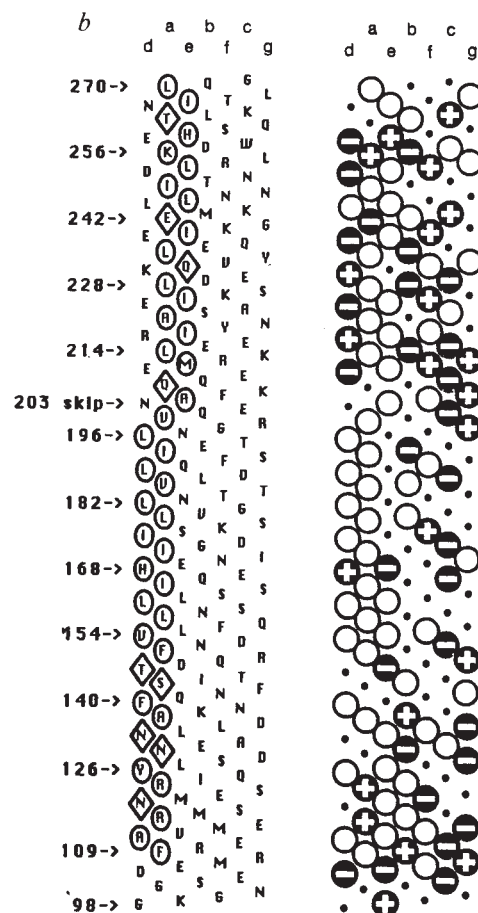
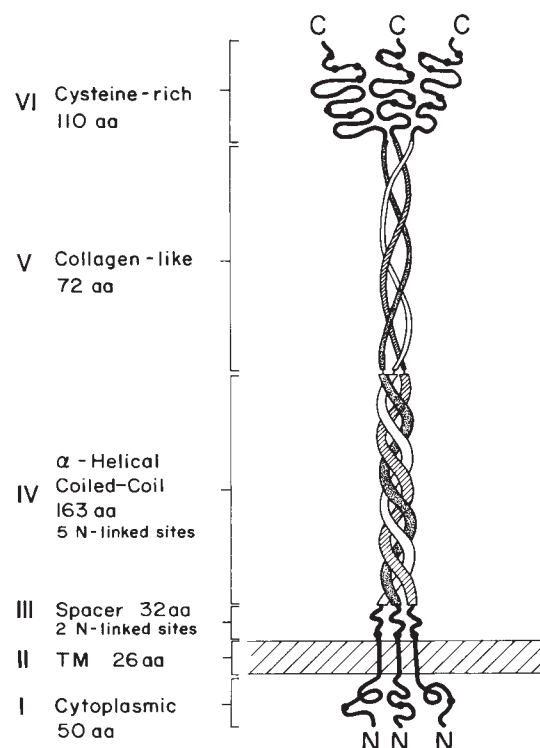


FIG. 6 Schematic model of the predicted trimeric structure of the type I bovine scavenger receptor. Small filled circles in the cytoplasmic (domain I), spacer (domain III) and cysteine-rich (domain VI) domains represent the approximate positions of cysteine residues. Each monomer contains a single membrane-spanning domain (domain II). This model assumes that the α -helical coiled-coil domain IV forms a single triple-stranded left-handed superhelix that merges with the right-handed collagen-like triple helix of domain V to form a single long fibrous stalk which would extend ~ 400 Å. Alternative models based on the aggregation of subunits into higher-order oligomers are described in the text. Abbreviations: aa, amino acids; TM, transmembrane.



homotrimer or heterotrimer. Transfection of plasmid pXSR7 into COS cells was sufficient for receptor activity, indicating that the homotrimer is functional. Our discovery of a second, truncated form of the scavenger receptor, type II (ref. 14), raises the possibility that heterotrimers could form *in vivo*.

A novel characteristic of the scavenger receptor is its unusual binding specificity for a broad, but limited, set of ligands, including some highly or abnormally negatively charged molecules⁶. Analysis of the type II form of the receptor¹⁴ indicates that one or both of the fibrous domains (IV and V), rather than the cysteine-rich domain (VI), determine the distinctive specificity of the receptor. In this regard, it is striking that collagens, which mediate a wide variety of cellular functions³³, can bind molecules such as von Willebrand factor³⁴, lipid parti-

cles in aortic intima³⁵, cholesteryl esters in tendon xanthomata³⁶, and native and modified LDL *in vitro*³⁷.

The physiological and pathophysiological functions of the type I and type II scavenger receptors are not known. Interest in the scavenger receptor has been focused mainly on its possible role in macrophage lipoprotein metabolism and atherosclerosis. Because of its broad binding specificity and collagen-like domain, it could also be involved in other physiological systems, including macrophage-associated immune responses and inflammation, and cell-to-cell or cell-to-extracellular matrix interactions. The isolation and characterization of cDNA clones for both forms of the scavenger receptor provide experimental tools that should be useful in studying the structure and function of this receptor. □

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Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels

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Mutations in the *period* (*per*) gene of *Drosophila melanogaster* affect both circadian and ultradian rhythms. Levels of *per* gene product undergo circadian oscillation, and it is now shown that there is an underlying oscillation in the level of *per* RNA. The observations indicate that the cycling of *per*-encoded protein could result from *per* RNA cycling, and that there is a feedback loop through which the activity of *per*-encoded protein causes cycling of its own RNA.

CIRCADIAN rhythms influence many behaviours and physiological processes. These rhythms are generated by an endogenous circadian 'clock', persist ('free-run') under constant environmental conditions, and respond to environmental time cues. In *Drosophila melanogaster*, two well-studied phenom-

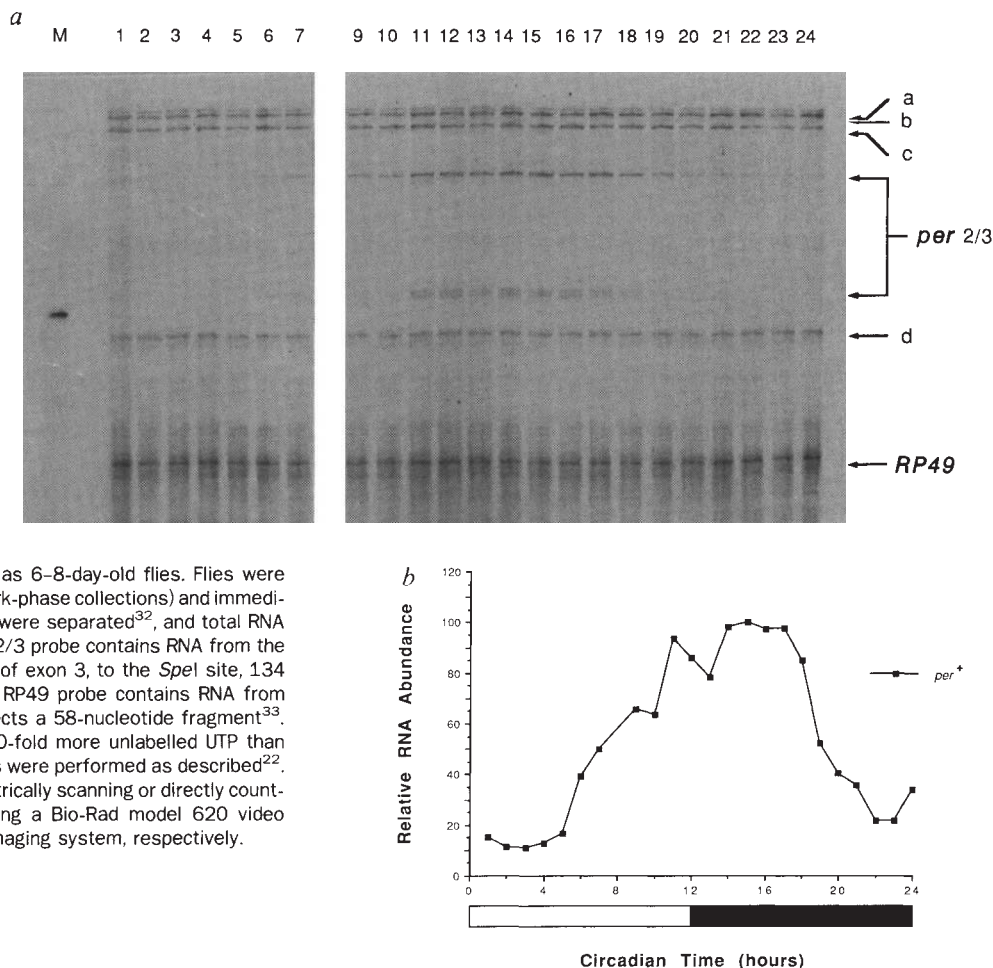
ena—eclosion and adult locomotor activity—are under the control of the circadian clock¹.

The product of the *period* (*per*) gene in *D. melanogaster* is a candidate clock molecule¹⁻³. The original three mutations described at the *per* locus either shorten (*per^S*), lengthen (*per^{L1}*) or essentially abolish (*per^{O1}*) circadian activity⁴. The *per* gene has been cloned and extensively analysed^{1,5}. *In situ* hybridization and immunohistochemical analyses indicate that *per* is expressed in numerous adult tissues, including the eyes, antennae, lateral brain neurons and putative glia in heads, and the salivary gland, ovaries and gut in bodies^{3,6,7}. Because the circadian oscillator has been mapped to the head⁸, we consider that the *per* expression most relevant to clock function is located here.

In the adult visual system, the immunoreactivity of *per* gene product (Per protein) fluctuates²; intense staining is readily detectable in photoreceptor nuclei in the middle of the night, but essentially no staining is detectable in the middle of the day. The staining fluctuations persist in constant darkness. These

FIG. 1 Levels of *per*⁺ cycle in flies under LD cycle conditions. *a*, RNase protections of RNA from flies collected during LD cycles. The number of hours after lights were turned on that the flies were collected are indicated above each RNase protection lane; lights were turned off after 12 h. M denotes the lane containing labelled 123-base pair markers (BRL); *per* 2/3 denotes the protected fragments from *per* RNA covering parts of exon 3 (upper) and exon 2 (lower). Bands a-d are due to incomplete digestions and full-length protections of the two probes by DNA. *b*, Quantitation of data shown in *a*. Relative RNA abundance refers to the values of *per*/RP49, where the peak value was adjusted to 100. The white and black bars represent lights on or off, respectively.

METHODS. Wild-type (Canton-S) adults (3–5-days-old) were exposed for 3 days to LD cycles before being collected as 6–8-day-old flies. Flies were then collected (in complete darkness for dark-phase collections) and immediately frozen on dry ice. Heads and bodies were separated³², and total RNA was extracted from the heads²². The *per* 2/3 probe contains RNA from the *Bgl*III site, 259 nucleotides from the start of exon 3, to the *Spe*I site, 134 nucleotides from the end of exon 2. The RP49 probe contains RNA from the *Hind*III site to the *Pvu*II site and protects a 58-nucleotide fragment³³. The RP49 probe was transcribed using 20-fold more unlabelled UTP than the *per*-specific probes. RNA hybridizations were performed as described²². Quantitation was done by either densitometrically scanning or directly counting the *per* exon 3 and RP49 bands using a Bio-Rad model 620 video densitometer or an AMBIS radioanalytic imaging system, respectively.



observations have recently been extended to show that there is only one peak of staining per day, that there are similar circadian fluctuations of Per immunoreactivity in additional adult central nervous system locations and that the various *per* mutations affect the antigen cycling in the same way as they affect behaviour³.

Here we show that *per* messenger RNA levels in the fly head also undergo circadian fluctuations during both 12-h light/12-h dark (LD) cycles and constant darkness (DD), and that *per* mutations affect the cycling of their own mRNA. The observations indicate that the oscillations in Per immunoreactivity could be due to oscillations in Per protein levels, which, in turn, are due to changes in *per* mRNA levels. They also indicate that Per protein activity is involved in a feedback loop that influences

the cycling of its own mRNA. We suggest that this feedback loop is a central feature of the circadian clock.

Analysis of *per*⁺ RNA levels during LD cycles

Analysis of Per protein immunoreactivity in heads has shown that there is a stronger signal in the middle of the night than in the middle of the day^{2,3}. To determine whether there is a similar fluctuation in *per* mRNA, we froze entrained wild-type (Canton-S) flies at different circadian times (CTs), extracted total RNA from heads and assayed for systematic changes in *per* mRNA levels.

RNA fragments protected by *per* antisense RNA covering parts of exons 2 and 3 (labelled *per* 2/3) fluctuated in abundance during LD cycles (Fig. 1a). These two exons are present in their entirety in the major (type A) and minor (types B and C) *per* RNAs³. Levels of *per* RNA were low when we turned lights on, started to increase about 5 h later, and peaked at about the time that we turned lights off. Maximum levels were maintained for 5–8 h before falling to minimum levels 1–2 h before we turned

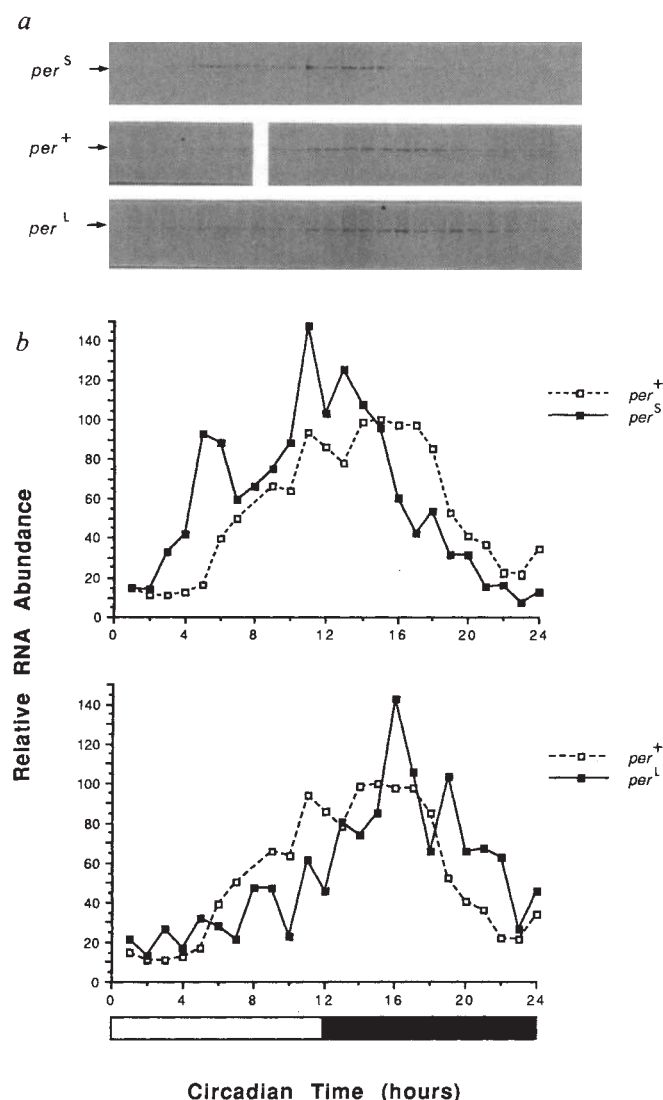


FIG. 2 Altered fluctuations of *per*^S and *per*^{L1} RNA under LD cycle conditions. **a**, Time courses of exon 3-protected fragments from *per*^S, *per*⁺ and *per*^{L1}. Arrows point to the exon 3 *per* fragment. The times at which these collections were taken (in hours after the last time lights were turned on) are shown under the graphs in **b**. **b**, Quantitation of data shown in **a**. Relative RNA abundance and circadian time are as described in Fig. 1. The peak level of *per*⁺ was set to 100. Both *per*^S (top graph) and *per*^{L1} (bottom graph) levels were adjusted relative to *per*⁺ levels by comparing *per*/*RP49* ratios averaged from three peak time points. □, *per*⁺ RNA levels; ■, *per*^S RNA levels (top graph) or *per*^{L1} RNA levels (bottom graph). **METHODS.** The *per*^S and *per*^{L1} flies were descendants of the original short (~19-h) and long (~29-h) period mutants⁴. Both *per*^S and *per*^{L1} flies were exposed to the same light-dark cycles as in Fig. 1. Fly collections, RNA and probe preparations, and RNase protections were as in Fig. 1.

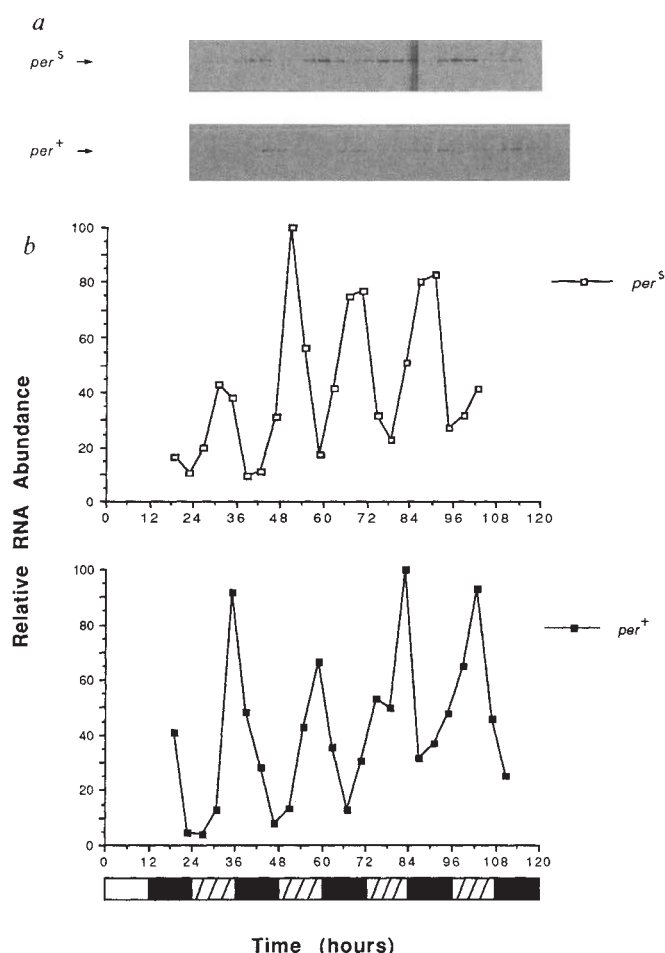


FIG. 3 The *per*^S and *per*⁺ RNAs cycle with different periods under DD conditions. **a**, Protection of exon-3 fragments with time. Arrows point to the exon 3-protected fragments. These protections can be compared with those of the light regime shown at the bottom of **b**, where the white box indicates the last period when lights were on, the black boxes indicate when lights were off, and the hatched boxes indicate when the lights would have been on had the LD cycle been continued. **b**, Quantitation of data. Relative RNA abundance is as described in Fig. 1. Peak levels for both *per*^S (□) and *per*⁺ (■) are set at 100. Time refers to the time after the start of the last period when lights were on.

METHODS. The *per*^S and *per*⁺ flies were exposed to three complete LD cycles and then kept in complete darkness as a continuation of the dark phase of the fourth LD cycle. Flies were collected every 4 h starting 19 h after the start of the last period when lights were on and ending 84 h (*per*^S) and 92 h (*per*⁺) later. All collections and RNA analyses were as in Fig. 1.

lights on. Quantitation of these data shows an amplitude (peak-to-trough ratio) of 5 to 10-fold (Fig. 1b). We replicated these measurements several times using RNAs from different fly collections, and consistently obtained amplitudes of 5 to 10-fold (data not shown). In addition, and to ensure that full-length *per* RNA was being assayed, we obtained identical results (although with fewer time points) with an RNase protection probe covering exon 7 and part of exon 8, and by northern blot analysis of poly(A)⁺ head RNA (data not shown).

Levels of *per^S* and *per^{L1}* RNA during LD cycles

Two *per* mutations, *per^S* and *per^{L1}*, are single missense mutations of the *per* gene^{10,11}. In addition to their effects on locomotor activity rhythms during DD cycles^{3,12}, these mutations also alter locomotor activity rhythms during LD cycles. Although mutant flies are rhythmic with a period of ~24 h under these conditions, the 'dusk' activity peaks of *per^S* and *per^{L1}* mutants occur earlier and later, respectively, than the wild-type *per⁺* dusk peak (M. Hamblen-Coyle and D. A. Wheeler, unpublished observations).

To determine whether *per* RNA cycling is altered in *per^S* and *per^{L1}* mutants, we performed RNase protections using adult head RNAs from flies collected during LD cycles.

Like *per⁺* RNA, both *per^S* RNA and *per^{L1}* RNA levels displayed clear rhythmic fluctuations (Fig. 2). The patterns are very similar to that of *per⁺*, but the *per^S* pattern is shifted to earlier times and the *per^{L1}* pattern to later times relative to the *per⁺* pattern. Both the peak levels of *per^S* and *per^{L1}* RNA and their 5- to 10-fold cycling amplitudes are indistinguishable from those of *per⁺* RNA (Fig. 2b, and data not shown).

Levels of *per⁺* and *per^S* RNA during DD cycles

The above results indicate that head *per* RNA levels cycle under LD cycle conditions. If this RNA cycling is like locomotor activity rhythms and not a driven rhythm (requiring the alternating light-dark cycles of the external environment), it should persist under free-running conditions. To test this prediction, we performed RNase protections on *per⁺* and *per^S* head RNA obtained from flies under DD cycle conditions.

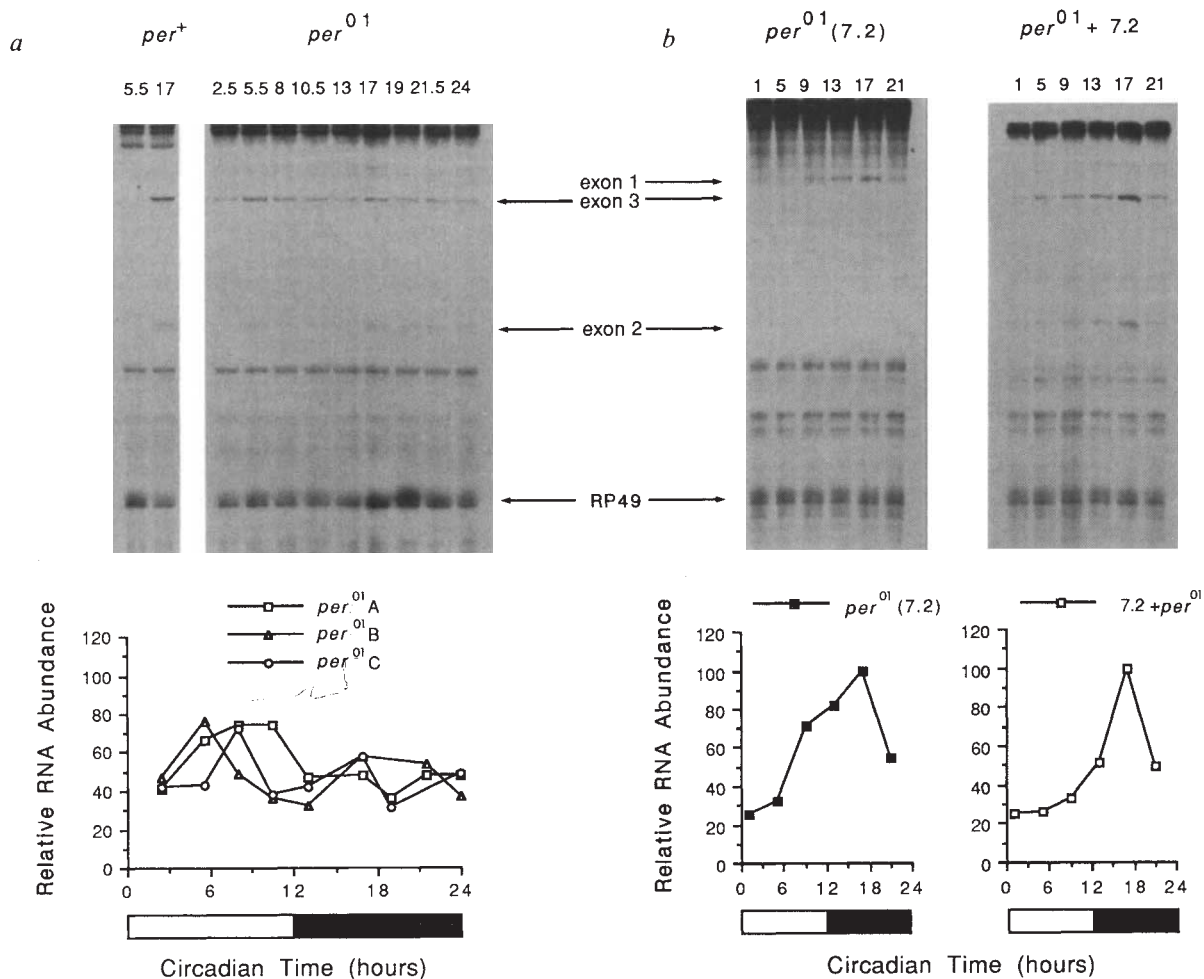


FIG. 4 A truncated *per* gene rescues the cycling of *per^{O1}* RNA. **a**, RNase protection of *per^{O1}* and *per⁺* RNAs. Times above each lane indicate the collection time, in hours, after lights were turned on. Arrows denote the positions of the *per* exon 3, *per* exon 2, and RP49 protected fragments. The two lanes under the *per⁺* label and nine lanes under the *per^{O1}* label contain RNase protections of wild-type and *per^{O1}* head RNAs, respectively. A, B and C denote three independent *per^{O1}* experiments. The level of *per^{O1}* RNA is expressed as a proportion of the peak level of *per⁺* RNA, which is set to 100 (see Fig. 1). Relative RNA abundance and circadian time are as in Fig. 1. **b**, RNase protections of a behaviourally rescued *per* germline transformant. Times above each lane indicate the collection time, in hours, after lights were turned on. RNase protections of *per^{O1}* RNA from *per^{O1}(7.2.9)* germline transformants using the exon 1 probe are referred to as *per^{O1}(7.2)*; *per^{O1} + 7.2* refers to RNase protections of both the *per^{O1}* and the 7.2-kb

RNAs from the *per^{O1}(7.2.9)* germline transformants using the exon 2/3 probe. Arrows denote the positions of protected fragments from *per* exon 1, *per* exon 2, *per* exon 3 and RP49. (In some lanes, for example, lanes 17 and 19, there is a detectable smudge, just above the RP49 band; this did not interfere with quantitation of the RP49 protected fragment.) Relative RNA abundance and circadian time are as in Fig. 1. The peak levels of *per^{O1}* (7.2) and 7.2 + *per^{O1}* were set to 100.

METHODS. The *per^{O1}* flies were descendants of the original arrhythmic strain⁴. Wild-type and *per^{O1}* flies were exposed to the same light-dark regimes, collection procedures, and analyses as in Fig. 1. In addition, a *per* probe specific for exon 1 was used. This probe was transcribed from a *Pst*I site, 348 nucleotides from the start of exon 1, to a *Sal*I site, 28 nucleotides from the start of exon 1 (ref. 9).

Over 4 days, we observed four peaks in the level of *per*⁺ RNA. The data indicate a period of ~24 h (Fig. 3a), indistinguishable from the average locomotor activity rhythm of wild-type flies measured during DD cycles^{1,13}. These peaks were in phase with the LD peak shown in Fig. 1, although the last peak occurred 4 h earlier than predicted from an exact 24-h rhythm. The amplitude, initially similar to the LD value (Fig. 1), decreased noticeably with time. This was due to a 2- to 3-fold increase in the trough RNA level (Fig. 3b).

The *per*^S RNA also cycled under DD cycle conditions, with each successive peak occurring earlier than its *per*⁺ counterpart (Fig. 3a). By measuring the time between peaks, we could estimate a period of ~20 h (Fig. 3b). This period is close to the *per*^S periods obtained with behavioural measurements¹. As with the *per*⁺ RNA, the *per*^S RNA amplitude fell during the timecourse because of an increase in the trough RNA levels.

We also performed RNase protections on head *per*^{L1} RNA obtained from flies under DD cycle conditions. There was no discernible RNA rhythm in these flies (data not shown). This result could be related to the fact that the behavioural rhythms of *per*^{L1} mutants, as well as their protein immunoreactivity (in LD cycles), are weak^{3,14}.

RNase protection

The *per*^{O1} mutation is a nonsense mutation in the *per* gene at a codon corresponding to position 464 of the amino-acid sequence^{10,11}, and has little or no biological activity in flies under DD cycle conditions¹. Under LD cycle conditions, flies are rhythmic with 24-h periods, but the rhythmic behaviour is distinct from that of wild-type flies. Rhythmic activity in the mutant seems to occur largely in response to the rhythmically applied external light cues rather than in response to an endogenous circadian clock¹⁵⁻¹⁷. To determine if *per*^{O1} RNA cycles, we used an LD-cycle timecourse of head *per*^{O1} RNA for RNase protections. Levels of *per*^{O1} RNA showed some variation (≤ twofold) but no consistent rhythmic fluctuations; the level of *per*^{O1} RNA was ~50% of that of *per*⁺ RNA at its peak (Fig. 4a). These data indicate that the *per*^{O1} flies contain a non-cycling median level of head *per* RNA. We cannot exclude the possibility, however, that in individual flies the cycling of *per*^{O1} RNA occurs, but that in the *per*^{O1}-mutant fly population it is not synchronized.

Germline transformation of arrhythmic *per*^{O1} flies with wild-type *per* DNA can restore rhythmic activity^{9-11,13,17-19}. We exploited this observation to test whether the *per*^{O1} transcript undergoes circadian cycling in a rhythmic strain. To facilitate the analysis, we used a strain in which the *per*^{O1} RNA is distinguishable from the transcript derived from the transformed *per* DNA. This strain (*per*^{O1(7.2:9)}) carries a transformed 7.2-kilobase (kb) piece of *per* DNA that contains all of the *per* coding DNA but is missing much of the noncoding DNA including exon 1. It has weaker locomotor activity rhythms than the *per*⁺ strain, with periods of 25-26 h¹³.

Results of protections using the *per* 2/3 probe, which does not distinguish between the RNAs derived from the 7.2-kb fragment and *per*^{O1}, showed that *per* RNA levels fluctuated with an amplitude of ~4 (Fig. 4b). The phase is indistinguishable from that observed with wild-type flies. To examine specifically the *per*^{O1}-derived transcript, we used a probe specific for exon 1. Analysis of the same *per*^{O1(7.2:9)} RNA samples showed that the *per*^{O1} RNA also cycles with an amplitude of ~4. We have also observed a similar but lower amplitude cycling of *per*^{O1} RNA in another independent transformed line, *per*^{O1(14.6:21)}^{13,18} (data not shown). We conclude that an active *per* gene rescues cycling of *per*^{O1} RNA as well as locomotor activity rhythms of the arrhythmic host strain.

Implications of *per* RNA cycling

The abundance of *per* RNA from adult heads fluctuates about 10-fold during a light-dark cycle in wild-type flies. We have

observed similar results by analysing separately eye RNA and brain RNA (data not shown).

There are several likely reasons why this dramatic cycling was not noted in previous studies^{20,21}. First, RNA was isolated from whole flies. The cycling amplitude is much lower for body RNA than for head RNA; as a consequence, the cycling amplitude of RNA isolated from whole flies is considerably lower than that of RNA isolated from heads. Second, most of our previous cycling experiments were performed under DD cycle conditions, where the RNA cycling is less dramatic. Third, the abundance of a transcript termed the '0.9-kb RNA' fluctuates more than 20-fold over the course of a day²⁰. It has now been shown that apparent differences in 0.9-kb RNA levels were due to developmental gating during the process of eclosion; the abundance of this RNA does not cycle as a function of circadian time²².

Although the results described here indicate that the *per* RNA fluctuations underlie the previously observed Per protein changes, the mutant analyses and the *per*^{O1} rescue experiment show that the reverse is also the case, that is, that Per protein levels or activities, or both, also affect the *per* RNA cycling. These observations are most easily explained by postulating that feedback of the *per* gene product regulates its own mRNA levels

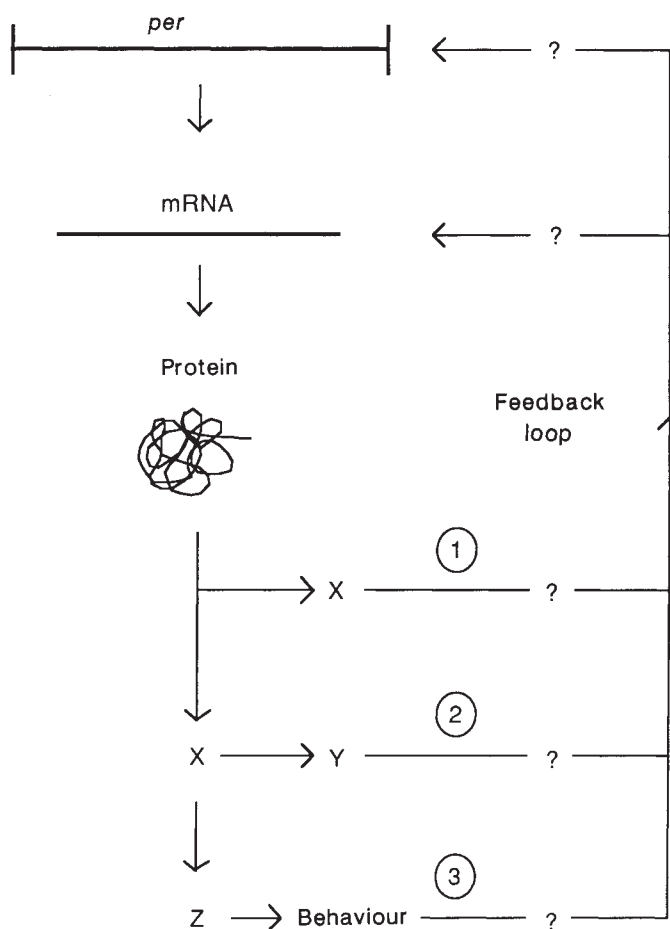


FIG. 5 Per protein feedback regulates the level of *per* RNA. The *per* gene (top thick line) is transcribed to yield *per* RNA (lower thick line) which is then translated to give Per protein (tangled line). X represents the result (unknown) of *per* activity, which is increased in *per*^S and decreased in *per*^{L1} mutants, as shown by Smith and Konopka³⁴. The Per protein could feed back (1) directly, (2) through intermediate biochemical steps (represented by Y); or (3) through the behaviour of the animal, to regulate *per* RNA levels. Z represents additional components that could lie in the pathway between *per* activity and behaviour. The cycling of *per* RNA could be effected by transcriptional or post-transcriptional feedback, depicted by arrows pointing back to the gene and the mRNA, respectively.

(Fig. 5). We do not know at what level this regulation occurs (transcriptional or post-transcriptional), nor do we know whether the effect of *per* activity is direct or indirect. An indirect feedback loop could even include some aspect of rhythmic behaviour, as all of the experimental manipulations we have used so far affect locomotor activity and RNA cycling in parallel.

There is strong evidence that *per* gene activity influences the function of the circadian clock^{1,4,5}. We suggest that the feedback loop described here is also an important component of the

circadian pacemaker. The fluctuating protein levels are a consequence of, and contribute to, the fluctuating RNA levels. Both of these fluctuations underlie the behavioural and biochemical oscillations characteristic of circadian rhythms. Although *per* RNA cycling could be associated with the clock output pathway (as is probably the case in other systems where specific RNA levels cycle in a circadian manner²³⁻³¹), the existence of such a feedback mechanism could make it difficult to distinguish clearly between clock function and clock output. □

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LETTERS TO NATURE

Young ages of two diogenites and their genetic implications

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THE achondrite meteorites known as eucrites and diogenites are thought to be igneous rocks derived from the crust and mantle, respectively, of a common parent planet or asteroid^{1,2}. Several eucrites have been dated at 4.5-4.6 Gyr by the Rb-Sr method⁴⁻⁶, and a Rb-Sr age of 4.45 ± 0.18 Gyr ($\lambda = 1.42 \times 10^{-11} \text{ yr}^{-1}$) has been reported³ for three diogenites. From these data, one cannot judge whether diogenites and eucrites are the same age. Here we present precise Rb-Sr data for two diogenites (Tatahouine and Johnstown) that yield an age of 4.394 ± 0.011 Gyr—significantly younger than our age of 4.52 ± 0.15 Gyr for the Juvinas eucrite. The low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of these diogenites, and their rare-earth element abundance patterns, are inconsistent with the interpretation that the young ages reflect secondary metamorphism. We suggest that our data raise doubts about a genetically close relationship between eucrites and these diogenites, and that there exist groups of diogenites that are genetically distinct from one another. Our results also suggest that igneous activity in the assumed parent body continued for about one hundred million years after its formation 4.52 Gyr ago. The parent body may thus have had a complicated evolutionary history.

Here we consider only the dates obtained using the Rb-Sr method so that we may discuss the relative ages of the meteorites irrespective of the accuracy of the decay constant of the parent nuclide. Strontium isotope ratios were measured with a VG-354 multi-collector mass spectrometer and the REE, Sr and Rb abundances were determined by the mass-spectrometric

isotope-dilution method using a JEOL JMS-05RB mass spectrometer.

The ^{87}Rb - ^{87}Sr analytical data and the Rb-Sr dates are given in Tables 1 and 2, respectively. Total blanks of Rb and Sr for 500-900-mg samples were 0.008 and 0.015 ng respectively, and because they never exceeded 0.05% of the sample values we did not make blank corrections. The Johnstown diogenite consists of coarse-grained clasts (centimetre-sized) and brecciated matrix. According to electron-probe microanalysis the coarse-grained clasts were orthopyroxene (opx; $\text{En}_{73-74}\text{Fs}_{23-24}$, where En is enstatite and Fs is forsterite) and the brecciated matrix contains mainly fragments of orthopyroxene, the composition of which is in the same range as that of the clasts. We analysed four samples from Johnstown: one sample containing several clasts and three samples from the brecciated portion. The Juvinas eucrite was separated into three fractions with heavy liquids (acetone, bromoform and methylene iodide saturated with iodine).

As shown in Table 2, the eight diogenite samples (four from Tatahouine and four from Johnstown) define an age of 4.394 ± 0.011 Gyr, with quite a small uncertainty. The isochron for these samples is shown in Fig. 1. We note that the data of Tatahouine-1 and -2 lie very close together, as do the data of Tatahouine-3 and -4, but that they are different from one another. Also, the data points for the Johnstown samples lie far from those for the Tatahouine samples, but all the diogenite data is described by a single isochron. The dotted line in Fig. 1 is the isochron for the Juvinas eucrite, corresponding to an age of 4.52 Gyr. The age of Juvinas has an uncertainty of 0.15 Gyr (see Table 2) so that the lower limit is 4.37 Gyr. As previous workers have reported⁴⁻⁶, the Rb-Sr ages obtained for Juvinas and other typical eucrites are in the range 4.51-4.54 Gyr (with uncertainties of 0.04-0.05 Gyr) so it is unlikely that the age of Juvinas lies in the range 4.37-4.39 Gyr. Given the ages of the Tatahouine and Johnstown samples (4.39 ± 0.01 Gyr), these diogenites are almost certainly younger than the eucrites. Birck and Allègre³ reported that the Rb-Sr system of Tatahouine was disturbed on a small length scale. We do not believe that our results reflect such a

TABLE 1 Rb-Sr analytical data

Sample	Amount (mg)	Sr (p.p.m.)	Rb (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}^*$	$^{87}\text{Rb}/^{86}\text{Sr}^\dagger$
Juvinas whole rock	18.61	69.01	0.0965	0.699229 ± 16	0.00404
Suspension‡	21.54	90.51	0.2262	0.699453 ± 16	0.00740
$d < 2.9 \text{ g cm}^{-3}$	15.07	168.97	0.1172	0.699094 ± 20	0.00201
$d > 3.3 \text{ g cm}^{-3}$	38.92	14.37	0.0900	0.700161 ± 24	0.01811
Johnstown§ Opx clasts	180.24	0.4250	0.01932	0.707421 ± 28	0.1314
Matrix 1	98.66	2.773	0.02152	0.700395 ± 36	0.02243
Matrix 2	99.71	2.707	0.02183	0.700460 ± 24	0.02327
Matrix 3	95.83	2.129	0.02642	0.026425 ± 20	0.03588
Tatahouine-1	803.54	0.06190	0.02614	0.778255 ± 16	1.2301
Tatahouine-2	750.76	0.06081	0.02570	0.778226 ± 36	1.2310
Tatahouine-3	791.39	0.06546	0.01035	0.724603 ± 40	0.3973
Tatahouine-4	900.58	0.07682	0.01062	0.724658 ± 40	0.4005
Roda*	46.47	6.086	0.04514	0.700418 ± 24	0.02144
Yamato-791422	96.41	13.434	0.08370	0.700176 ± 20	0.01801
NBS 987	—	—	—	0.710195 ± 20	—

* 2σ uncertainties in the last two digits.

† Uncertainties are $<0.5\%$.

‡ Suspension (Juvinas) is the fraction suspended in acetone.

§ Johnstown, AMNH No. 2497.

|| Tatahouine-1 and -2, No. 1644.

¶ Tatahouine-3 and -4, Me 2651.

Roda, No. 640.

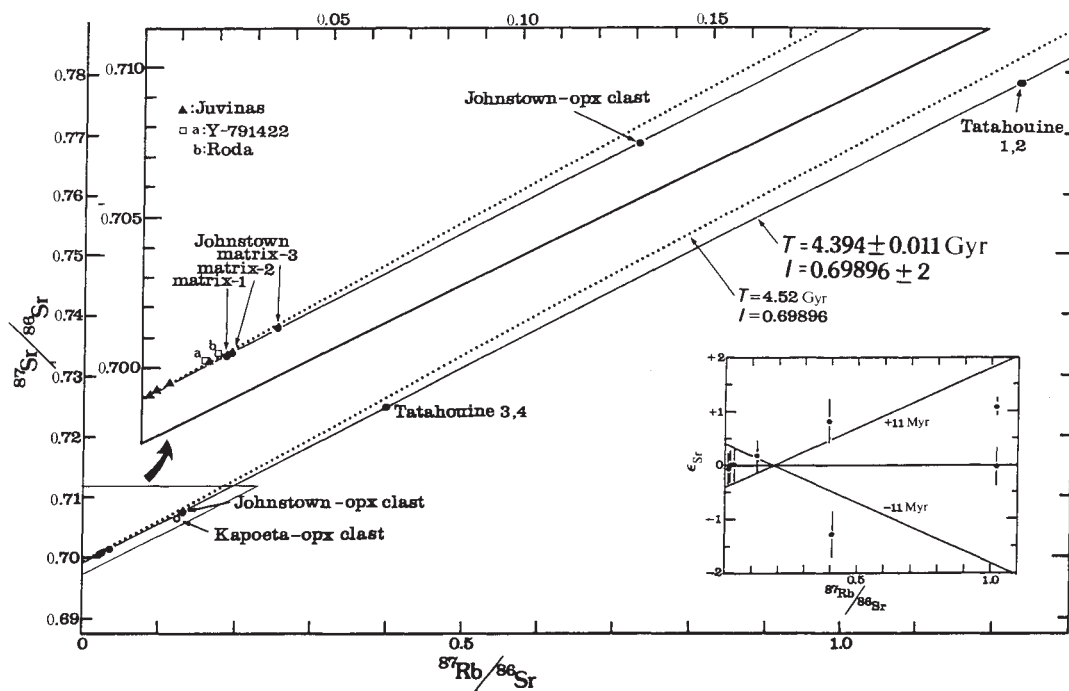
disturbance because our samples were larger ($\sim 1 \text{ g}$) and the data for the fragments of the same chip are in very good agreement with one another. On the other hand, the Rb-Sr ages for the Roda and Yamato-791422 (Y-791422) diogenites are close to the age of the eucrite (see Table 2). Y-791422 is classified as a Y-75032 type of diogenite. These diogenites are unique in that they have relatively calcium-rich compositions compared with other diogenites, and their mineralogical and chemical characteristics⁷ are similar to those of the eucrites. These observations indicate that diogenites may be roughly divided into two groups. The first group, to which the Tatahouine and Johnstown meteorites belong, are younger than eucrites, and the other group, to which Roda and Y-75032 type of diogenites belong, have the Rb-Sr systematics similar to those of eucrites. One interpretation of the young age of the Johnstown and Tatahouine diogenites is that it reflects secondary metamorphism (as a result of shock, for example) or an evolutionary process commencing from diogenites of the Roda or Y-75032 type.

Another interpretation is that the Tatahouine and Johnstown diogenites are genetically distinct from those of the Roda or Y-75032 type and from the eucrites. Based on the REE patterns and $^{87}\text{Sr}/^{86}\text{Sr}$ initial ratios discussed below, we prefer the latter interpretation.

Figure 2 shows that the REE contents of the Roda and Y-791422 diogenites (4.5-Gyr old) are higher than those of the Tatahouine and Johnstown samples and are similar to the pattern of the pyroxene fraction of the Juvinas eucrite, whereas the REE patterns of Tatahouine and Johnstown (4.394-Gyr old) have some unique characteristics, especially the concave curvature between lanthanum and samarium. Although the REE patterns for matrix samples of the Johnstown meteorite seem to be rather similar to those of the Roda sample, they are distinguished from one another on the Rb-Sr isochron plot. These REE patterns do not favour a simple formation model⁸ for diogenites, assuming that REE partition coefficients for orthopyroxene do not vary appreciably with conditions of

FIG. 1 The isochron plot for diogenites. The narrow triangular area from the lower left is shown magnified in the upper left. The data for fragments from the same chip (Tatahouine-1, -2, -3 and -4) are too close to distinguish on this plot. Opx, orthopyroxene. The inset on the right-hand side shows the relative deviation ($\times 10^{-4}$) from the best-fit line age of 4.394 Gyr ($I = 0.69896$) as a function of $^{87}\text{Rb}/^{86}\text{Sr}$.

$$\epsilon_{\text{Sr}} = \frac{(^{87}\text{Sr}/^{86}\text{Sr})_{\text{meas}} - (^{87}\text{Sr}/^{86}\text{Sr})_{\text{calc}}}{(^{87}\text{Sr}/^{86}\text{Sr})_{\text{calc}}} \times 10,000.$$



formation such as cooling rate temperature and/or chemical composition.

The possibility that the Tatahouine and Johnstown diogenites are genetically different from the eucrite group and some unique diogenites (Roda and Y-75032 type) is supported by the $^{87}\text{Sr}/^{86}\text{Sr}$ initial ratio of the isochron for the Johnstown and Tatahouine samples. If the ages of these samples indicated the age of secondary metamorphism, the isochrons of 4.394 Gyr and 4.52 Gyr would cross around the data point of Roda or Y-791422 in Fig. 1. Instead they cross near the $^{87}\text{Sr}/^{86}\text{Sr}$ axis, that is, the initial $^{87}\text{Sr}/^{86}\text{Sr}$ values of these isochrons are similar (they are in fact the same within the error limits). The initial $^{87}\text{Sr}/^{86}\text{Sr}$ value of the 4.39-Gyr isochron is 0.69896 ± 0.00002 and that of the 4.52-Gyr isochron is 0.69896 ± 0.00002 . The increase of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in the parental material should have been $<0.00002-0.00004$ over about one hundred million years. Thus, the Tatahouine and Johnstown diogenites were formed from material with an $^{87}\text{Rb}/^{86}\text{Sr}$ abundance ratio <0.004 , which corresponds to an increase of 0.00002 in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio over one hundred million years. In diogenitic materials the Rb/Sr abundance ratios are ≥ 0.006 (for example, 0.0064 in Y-791422) so Roda, Y-791422 or other such diogenitic materials cannot be the parent material. The parent material of basaltic achondrites is considered^{5,9} to have a low Rb/Sr abundance ratio ($^{87}\text{Rb}/^{86}\text{Sr} < 0.01$, corresponding to Rb/Sr < 0.0037) and such parent material may have remained after the formation of the eucrites and the diogenites of the Roda or Y-75032 type. Magmatic differentiation may have followed continuously in deep layers of the parent body to produce diogenites such as Johnstown or Tatahouine and possibly some of the young cumulate-type eucrites such as Sioux County⁶. The age of the Sioux

TABLE 2 Rb-Sr ages

Sample	Age (Gyr)	I_{Sr}^*
Tatahouine (4 samples)	4.398 ± 0.028	0.69895 ± 4
Johnstown (4 samples)	4.390 ± 0.035	0.69897 ± 4
Tatahouine and Johnstown (8 samples)	4.394 ± 0.011	0.69896 ± 2
Roda	4.63 ± 0.08	—
Yamato-791422	4.60 ± 0.07	—
Juvinas	4.52 ± 0.15	0.69896 ± 2

The ages for Roda and Y-791422 are the model ages calculated from their Rb-Sr data and the initial ratio of Juvinas, 0.69896.

* 2σ uncertainties in the last digit.

County eucrite is 4.19 ± 0.14 Gyr ($\lambda = 1.39 \times 10^{-11}$ was used for the calculation of the age from the gradient of its isochron; this age becomes 4.10 Gyr if $\lambda = 1.42 \times 10^{-11}$ is used) and the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio seems low, 0.69897 ± 8 . The REE patterns, however, are not easily reconciled with close genetic relationship between the young diogenites, such as Johnstown or Tatahouine, and eucrites. Our data are consistent with the hypothesis that eucrites such as Juvinas and diogenites such as Johnstown and Tatahouine came from different parent bodies. It is difficult, however, to suggest candidates that could be the complementary material to these 'young' diogenites. □

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Search for high-energy ions from fracture of LiD crystals

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RECENT experiments¹⁻⁵ have shown that the rate of 'solid-state' fusion of deuterons during either electrolysis or high-pressure deuteration of palladium or titanium must be at least many orders of magnitude lower than has been claimed⁶⁻⁸. It has been conjectured (refs 9-11; F. J. Mayer, J. S. King and J. R. Reitz, unpublished data; and R. Ryan *et al.*, unpublished data) that fusion, which would occur at vanishingly low rates under static conditions, might occur at detectable rates under dynamic conditions. In particular, if a metal lattice, embrittled by deuteration, were to undergo microfracturing, deuterons might be accelerated in the transient high electric fields across the cracks, reaching energies sufficient to cause fusion. Emission of low-energy ions has been observed during mechanical fracture of $\text{TiD}_{0.8}$ (H. O. Menlove *et al.*, unpublished data), and Klyuev *et al.*¹² claim, during fracture of LiD single crystals, to have detected an excess of neutrons above the cosmic-ray background. These results, and earlier claims to have observed electrification^{13,14} and emission of low-energy electrons^{15,16} and ions¹⁶ during fracture of dielectric crystals, prompted me to see whether energetic protons are emitted during

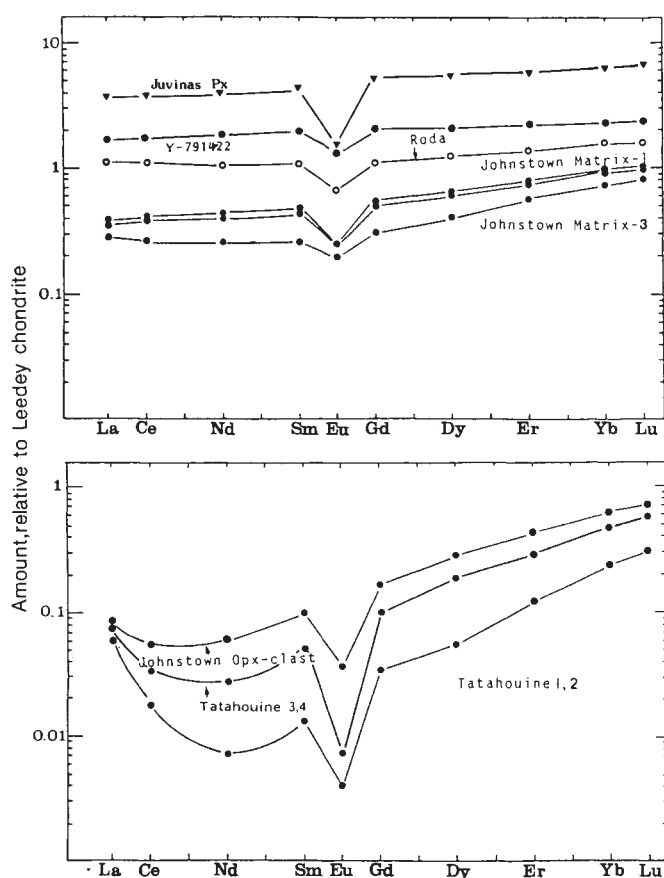


FIG. 2 The REE patterns of Tatahouine and Johnstown-opx are characterized by curvature between La and Sm. Such curvature has not been found in the REE patterns of the pyroxene fractions of eucrite.

fracture of LiD crystals. I used a CR-39 track-recording plastic detector to detect any such emitted particles. However, no particles were observed, casting doubt both on the claim of Klyuev *et al.* to have seen fusion neutrons and on the conjecture that deuterated palladium or titanium could produce a detectable number of energetic particles as a result of fusion at cracks.

Figure 1 shows the geometry of the experiment. I obtained a $\sim 15\text{-cm}^3$ single crystal of LiD (grown by M. Delong, University of Utah) and cleaved it vertically along (100) planes into ~ 100 thin plates. The bottom of the crystal was supported 0.9 mm above the surface of the CR-39 plastic detector, a negligible distance compared with the range in air of either a 3.03-MeV proton or a 1.01-MeV triton emitted in the fusion reaction $d+d \rightarrow p+t$. To eliminate background tracks from radon decay and cosmic-ray interactions, I etched the CR-39 and located pre-existing tracks just before cleaving the LiD crystal. In this way, as in a previous search for cold fusion in my laboratory⁵, the background was reduced to zero. After cleaving the crystal one hundred times, the CR-39 was re-etched and scanned for new tracks. There were nine pre-existing tracks of particles with atomic numbers 1 and 2, but no new tracks. The etching conditions were the same as in ref. 5.

Table 1 compares this search for charged particles from the reaction $d+d \rightarrow p(3.03\text{ MeV}) + t(1.01\text{ MeV})$ (42% branching ratio) with the search of Klyuev *et al.*¹² for neutrons from the reaction $d+d \rightarrow n(2.45\text{ MeV}) + {}^3\text{He}(0.80\text{ MeV})$ (58% branching ratio). Each fragment of the LiD single crystal used here was $\sim 2.5\text{ cm}$ long and $0.4\text{--}2.5\text{ cm}$ high, but the ranges of a 3.03-MeV proton and a 1.01-MeV triton in LiD are only $\sim 0.19\text{ mm}$ and $\sim 0.015\text{ mm}$, respectively, much shorter than the height of the cleavage crack. The tritons sample only $\sim 8\%$ of the area of crack that the protons sample and can be ignored. I have taken into account not only the solid angle and efficiency for detection but also the surface area of a crack from which protons could reach the detector and be recorded. To an excellent approximation, fusion protons and tritons would be emitted isotropically, because the velocity of the compound nucleus ${}^4\text{He}^*$ is negligible. It is also reasonable to assume that the fusion events take place uniformly throughout crack areas. The product of detection efficiency and effective source area, $\langle \eta A \rangle$, can be calculated by integrating over emission zenith angle and height above the bottom of the LiD crystal and then multiplying by

TABLE 1 Comparison of this experiment with that of Klyuev *et al.*¹²

	Klyuev <i>et al.</i> ¹²	This experiment
Effective source area per cleavage	$\sim 0.09\text{ cm}^2$	$\sim 0.04\text{ cm}^2$
Detection efficiency (including solid angle)	2.6%	12%
Number of cleavages	several	$\sim 10^2$
Particle studied	neutrons	protons and tritons
Branching ratio for reaction	0.58	0.42
channel sought		
Observed number of particles	0.26 ± 0.11 per fracture	0
No. expected, based on Klyuev <i>et al.</i>	—	54 ± 23

the crack length. At zenith angles greater than $\sim 40^\circ$, an etched track of a 3-MeV proton in CR-39 is recorded with decreasing efficiency. A maximum zenith angle of 40° was assumed, which leads to an efficiency $\eta = 0.12$ (that is, a solid angle of 12% of $4\pi\text{ sr}$); an effective height of 0.019 cm; and a crack length of 2.5 cm, the full horizontal length of the crystal. The result for these experiments, $\langle \eta A \rangle = 0.0057\text{ cm}^2$ for protons, is close to what would be obtained by doing the integral and taking into account the detailed dependence of track-recording efficiency on proton energy and zenith angle, as discussed in ref. 5.

In the experiment of Klyuev *et al.*, the number of neutrons claimed to have been detected was 0.26 ± 0.11 per cleavage crack of area 0.09 cm^2 . Taking into account the efficiency of their detector (2.6%), one infers a neutron production rate of 110 per cm^2 of crack surface. Comparing the numbers for the neutron experiment and my proton experiment, and taking into account the relative production rates of protons and neutrons in $d\text{-}d$ fusion, I expected to detect 54 ± 23 protons per 100 cleavages, but I found none. (The uncertainty is due to the uncertainty in average neutron production rate given in ref. 12.) At 90% confidence level, my null result for protons allows me to infer that the neutron production rate in LiD should be less than 4 per cm^2 of crack surface.

I use this limit to test the conjecture that $d\text{-}d$ fusion might occur in the transient electric fields across a propagating crack in TiD_2 or PdD_x . First, note that transition-metal deuterides are bonded differently from LiD, which leads to differences in properties¹⁷. The former have metallic properties: the deuterons are mobile, the electrical conductivity is as high as that of a metal, and there is some plasticity (greater for palladium than for titanium, which is rather brittle for a metal). LiD has properties similar to those of metal halides: the bonding is ionic, cleavage is easily induced, cracks propagate readily, and electrical conductivity is low and results from cation vacancy migration with a high activation energy. The conductivity of LiD is only $\sim 10^{-23}\text{ }\Omega^{-1}\text{ cm}^{-1}$ at room temperature. For a conservative estimate, I make the rather extreme assumption that deuterated palladium or titanium undergoes brittle fracture as readily as does LiD and develops as large an electric field across a crack as does LiD. The upper limit for deuterated palladium or titanium then becomes four neutrons generated by fusion for every cm^2 of crack area formed. To attribute the origin of the neutron fluences in the original reports of cold fusion to a crack mechanism would require the creation of $\sim 10^{10}\text{ cm}^2$ (ref. 6) and $\sim 10^5\text{ cm}^2$ (ref. 7) of crack area. To account for even the lowest claimed cold-fusion yield, $\sim 10^3$ neutrons cm^{-3} during an 18-h run (H. O. Menlove *et al.*, unpublished data), at least 10^4 cm^2 of crack area would have to be generated. These authors claimed that the neutrons often occurred in bursts of up to 250 neutrons, which would require the creation of $>2,500\text{ cm}^2$ of crack area per neutron burst. Although this might conceivably occur in TiD_2 , it seems extremely unlikely in PdD_2 , in which plastic flow develops when it is mechanically deformed.

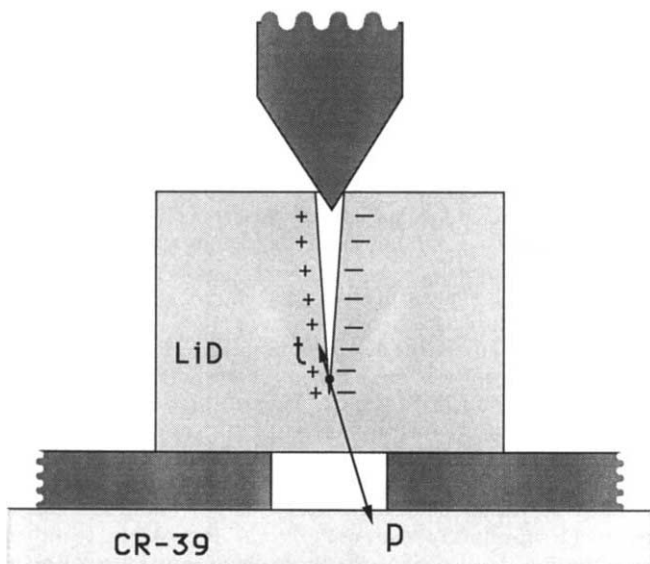


FIG. 1 System for recording tracks of protons and tritons from $d\text{-}d$ fusion resulting from the acceleration of deuterons in the transient electric field across a cleavage crack.

In a very recent report¹⁸, Klyuev and his collaborators claim to have detected neutrons emitted during the mechanical treatment of titanium shavings in the presence of deuterated substances. Almost certainly this claim is contradicted by my negative result. □

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Low-temperature long-range oxygen order in $\text{YBa}_2\text{Cu}_3\text{O}_z$

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OXYGEN ordering in $\text{YBa}_2\text{Cu}_3\text{O}_z$ is responsible for the tetragonal-to-orthorhombic phase transition at high temperature, and gives rise to additional ordered superstructures at low temperature^{1–3}. We suggest here that such ordered superstructures are intimately related to the ‘plateaus’ in the plot of transition temperature versus oxygen content that have been observed by various investigators^{4,5}. We present a consistent theoretical model for oxygen ordering in $\text{YBa}_2\text{Cu}_3\text{O}_z$ featuring a parameter-free phase diagram for this system and an algorithm for determining further ordered phases, the predicted structures and diffraction patterns of which agree closely with recent experimental findings^{2,3}.

Variations of oxygen content in $\text{YBa}_2\text{Cu}_3\text{O}_z$ are accommodated largely by changes of oxygen coverage in the ‘basal’ or ‘chain’ plane (the mirror plane located between barium ions). Oxygen configurational statistics can be modelled^{6,7} by an Ising model consisting of three interpenetrating square lattices, one being always occupied by copper ions and the other two being occupied by oxygen ions (Fig. 1). In the tetragonal (disordered) phase (T), both oxygen sublattices are occupied randomly by oxygen ions (Fig. 1a). In the orthorhombic 90-K superconducting phase (Ortho I, or OI for short, Fig. 1b), one sublattice is occupied entirely at stoichiometry $z = 7$ by oxygen ions, and the other sublattice is empty. The well-known O–Cu–O ‘chain structure’ is thereby obtained.

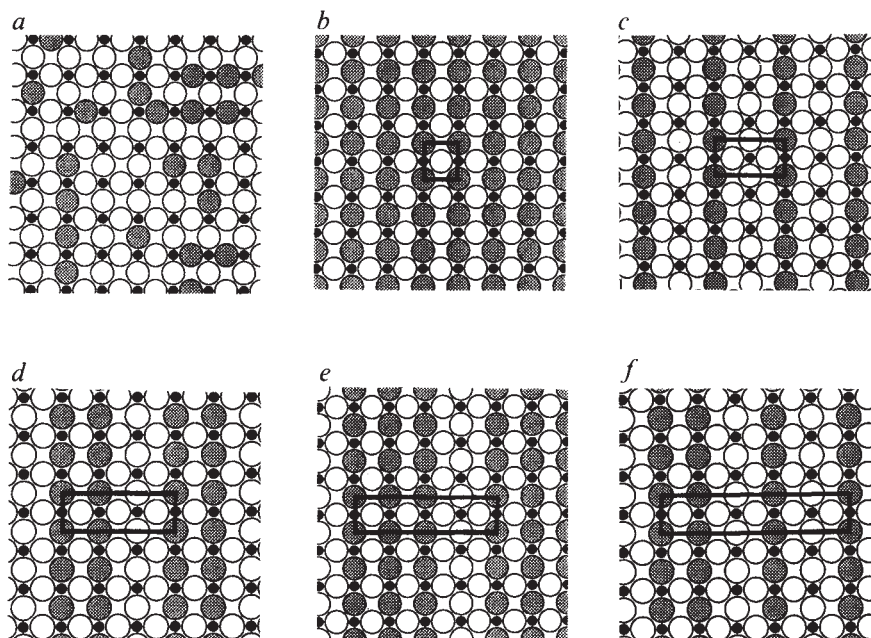
Many essential features of oxygen ordering in this system may be described by means of three effective pair interaction parameters: V_1 , which couples the two sublattices; V_2 , a negative (attractive) second-neighbour pair interaction which couples oxygen sites in the O–Cu–O chain direction; and an effective repulsive interaction, V_3 , which couples oxygen sites in the direction normal to the chains. We have used first-principles-

derived parameters⁸ V_1 , V_2 and V_3 to calculate⁹ the low-temperature portion of the phase diagram by means of a low-temperature modification (A. Finel, personal communication) of the cluster variation method¹⁰ (CVM). The resulting diagram, shown in Fig. 2, qualitatively resembles the one derived by Kikuchi and Choi¹¹. The index z in $\text{YBa}_2\text{Cu}_3\text{O}_z$ is related to c by $z = 2c + 6$, assuming that all vacant oxygen sites are located in the basal plane. Four phases are found to be stable for this set of interactions: the tetragonal (T) phase (Fig. 1a); the orthorhombic (OI) which, at low temperature and for $z \neq 7$, will differ from the perfect structure shown in Fig. 1b by having a few empty ($\square$ -Cu- $\square$, where $\square$ is a vacant O site) ‘vertical’ rows dispersed among the filled ones; the anti-Ortho I ($\overline{\text{OI}}$) phase, consisting of a distribution of filled rows among predominantly empty ones; and a phase with double-cell structure (or OII, Fig. 1c). The other structures depicted in Fig. 1 are stabilized by longer-range interactions, as explained below. All transitions are predicted by the CVM to be second-order except for the T–OII and $\overline{\text{OI}}$ –OII reactions.

Monte Carlo simulations¹² and CVM calculations⁹ of intrachain correlations show that for every oxygen concentration, very long O–Cu–O and $\square$ -Cu- $\square$ chains form at low temperatures. A one-dimensional Ising model is then appropriate to describe the states of quasi-one-dimensional interchain ordering. We now want to extend the range of effective interactions beyond that used to calculate the phase diagram of Fig. 2. As intrachain modulations have not been observed, the effective (negative) interaction V_2 need not be supplemented by longer-range interactions along the chains, as no new features will be introduced in the phase diagram. For fully formed chain states, all other pair interactions can be projected onto the direction normal to the chains. The resulting interactions are then between identical parallel chains, Cu facing Cu, O facing O, in principle out to arbitrary distance. Decay of these repulsive interactions is expected to be slow because of the low dimensionality of the interacting units. At low temperatures, we thus expect long-range repulsive interactions beyond V_3 along the a direction to have an important role. Such interactions, denoted here by $J_1 \equiv V_3$, J_2 , $J_3, \dots$, are expected to obey the convexity relation $J_n < \frac{1}{2}(J_{n+1} + J_{n-1})$, valid for Coulomb repulsion (screened or not). The ground states of quasi-one-dimensional order for such convex interactions are known^{13,14} and may be obtained exactly by a continued-fraction algorithm, shown to be completely equivalent to a structure-combination branching mechanism¹⁵. The branching algorithm is illustrated in Fig. 3a. The Ortho I and Ortho II structures (level I in Fig. 3a) are the generating structures for those shown at successive levels (II, III, IV, $\dots$), or ‘generations’. The level II structure is obtained by telescoping level I structure elements $\langle 1 \rangle$ and $\langle 10 \rangle$ to form structure $\langle 110 \rangle$ (Ortho III, Fig. 1d). At level III, $\langle 10 \rangle$ combines with $\langle 110 \rangle$ to form $\langle 11010 \rangle$ and $\langle 1 \rangle$ combines with $\langle 110 \rangle$ to form $\langle 1^30 \rangle$ (Ortho IV), the exponent indicating how many times the associated symbol must be repeated. In practice, this branching process continues to any level n at which the corresponding interaction J_n is large enough significantly to favour n th-level-ordered superstructures over random mixtures of $(n-1)$ th-level structures of same average oxygen concentration. Structures located on the leftmost branches of the tree have recently been proposed¹⁶ as transient, that is, metastable, states of order. Such structures, called ‘Magneli phases’ by analogy with those found in minerals, appear, in the present model, as a stable subset of the correct ground states for the convex interaction problem, that is, those of the branching algorithm.

In Fig. 3a, the stoichiometric composition is indicated below the structure formula for each of the ‘branching’ phases. Limiting concentrations, indicated on the vertical rises, are those at which the interchain pair correlations characteristic of one phase change to those of the adjacent one. The vertical scale is practically that of temperature, as branching to increasingly higher generations is expected to occur at successively lower tem-

FIG. 1 Examples of two-dimensional ordering of $\text{YBa}_2\text{Cu}_3\text{O}_z$ obtained by Monte Carlo simulation performed with parameters described in the text. Small filled circles indicate copper ions, large shaded circles denote oxygen ions and open circles denote vacant sites. *a*, Tetragonal (T) phase at $c=0.22$, high temperature; all other structures are for stoichiometric phases at low temperature; *b*, Ortho I ($\langle 1 \rangle$) in the notation of Fig. 3*a*; *c*, Ortho II ($\langle 10 \rangle$); *d*, Ortho III ($\langle 110 \rangle$); *e*, Ortho IV ($\langle 1^30 \rangle$); *f*, $\langle 11010 \rangle$. Unit cells of ordered superstructures are outlined; period lengths are 1, 2, 3, 4, 5 (in Ortho I lattice-parameter units) for *a*–*f*, respectively.



peratures. Branching phases should be observed mainly at oxygen content higher than OII stoichiometry ($c > 0.25$, $z > 6.50$), that is, between OII and OI. This is because, on the other side, between $\bar{\text{O}}\text{I}$ and OII, the tetragonal-to-orthorhombic transition (a prerequisite for stability of quasi-one-dimensional superstructures) occurs at very low temperatures. Scanning across the branching superstructures at the level of fourth generation, from $\langle 10 \rangle$ to $\langle 1 \rangle$, there are seven phases. The $(h00)$ sections of the corresponding ideal diffraction pattern are indicated in Fig. 3*b*. The diffraction patterns are obtained by taking the square of the Fourier transform (structure factor) of the sequence of 1's and 0's representing the structure. Intensities are normalized to unity for the fundamental peaks at (000) and (100).

It is instructive to compare the predictions of the model with recent experimental results, in particular the electron diffraction and micrograph studies of Beyers *et al.*³ and of Reyes-Gasga *et al.*². At room temperature, Beyers *et al.*³ observe a 'tweed' structure, characteristic of phase separation, for $z < 6.28$, then a wide Ortho II phase region up to about 6.61. On increasing the oxygen content, $h = 2/5$ diffraction spots are observed near $z = 6.65$, then $h = 0.37$ spots near $z = 6.71$, then $h = 1/3$ spots near $z = 6.75$, becoming broader near 6.80. Finally, diffuse streaks are observed around oxygen content 6.85, and no discernible diffuse intensity for $z > 6.85$. The theoretical results indicate that at ~ 300 K the diffraction patterns characteristic of the two-phase region and of the Ortho II structure should be observed for $c = 0.125$ – 0.15 and for c between 0.15 and the first branching phase, respectively. According to Fig. 3*b*, at the fourth-generation level the following features should be observed at higher oxygen concentrations: a structure with diffraction spots at $h = 3/4$ and $4/7$ near $c = 0.27$, then one with spots at $2/5$ and $3/5$, then one with spots at $3/8$ and $5/8$, then the 'Ortho III' phase with spots at $h = 1/3$ and $2/3$, then very broad diffuse intensity maxima near $2/7$ and $5/7$, then very flat, 'streaky' diffraction patterns characteristic of cell-quadrupling and cell-quintupling structures. Finally, for $c \geq 0.45$ ($z \geq 6.9$), diffuse intensity should no longer be discerned.

The agreement with the electron diffraction observations of Beyers *et al.*³ is almost perfect except for a difference between measured oxygen content and calculated oxygen stoichiometry, the former being systematically higher than the latter. This

discrepancy can be accounted for, however, if some loss of oxygen occurred in the electron-microscope column in the course of observation. The first detected³ branching phase has diffraction spots at $2/5$, $3/5$ rather than at $3/7$, $4/7$. However, the latter maxima may be difficult to resolve from the dominant Ortho II peak at $h = 1/2$. The theoretical model accounts for

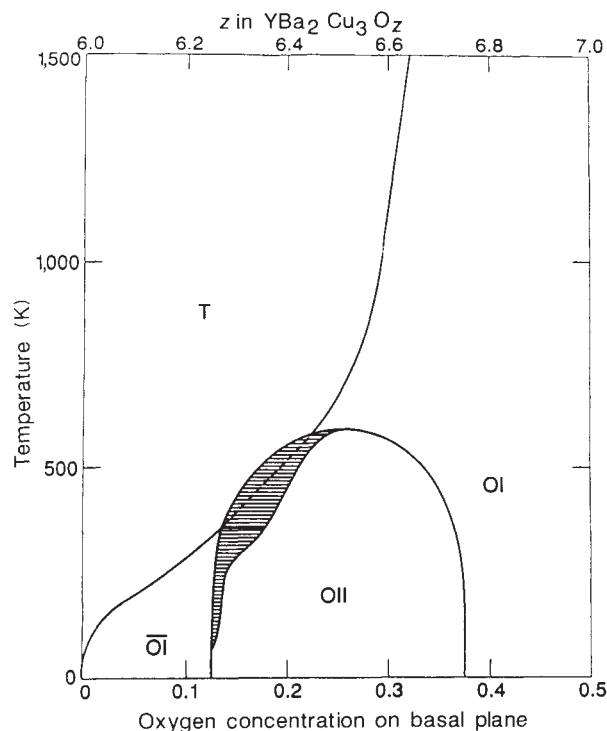


FIG. 2 Oxygen-ordering phase diagram of $\text{YBa}_2\text{Cu}_3\text{O}_z$ calculated by the cluster-variation method⁹ from parameters obtained by first-principles LMTO (Linear Muffin-Tin Orbital) calculations⁸: $V_1 = 6.9$ mRy, $V_2 = -2.4$ mRy, $V_3 = 1.1$ mRy. All transitions are second-order except where indicated by the shaded region. Horizontal lines symbolize tie lines in two-phase equilibrium regions. Lower oxygen-content scale denotes basal-plane coverage, c , upper scale denotes oxygen stoichiometric index $z = 2c + 6$.

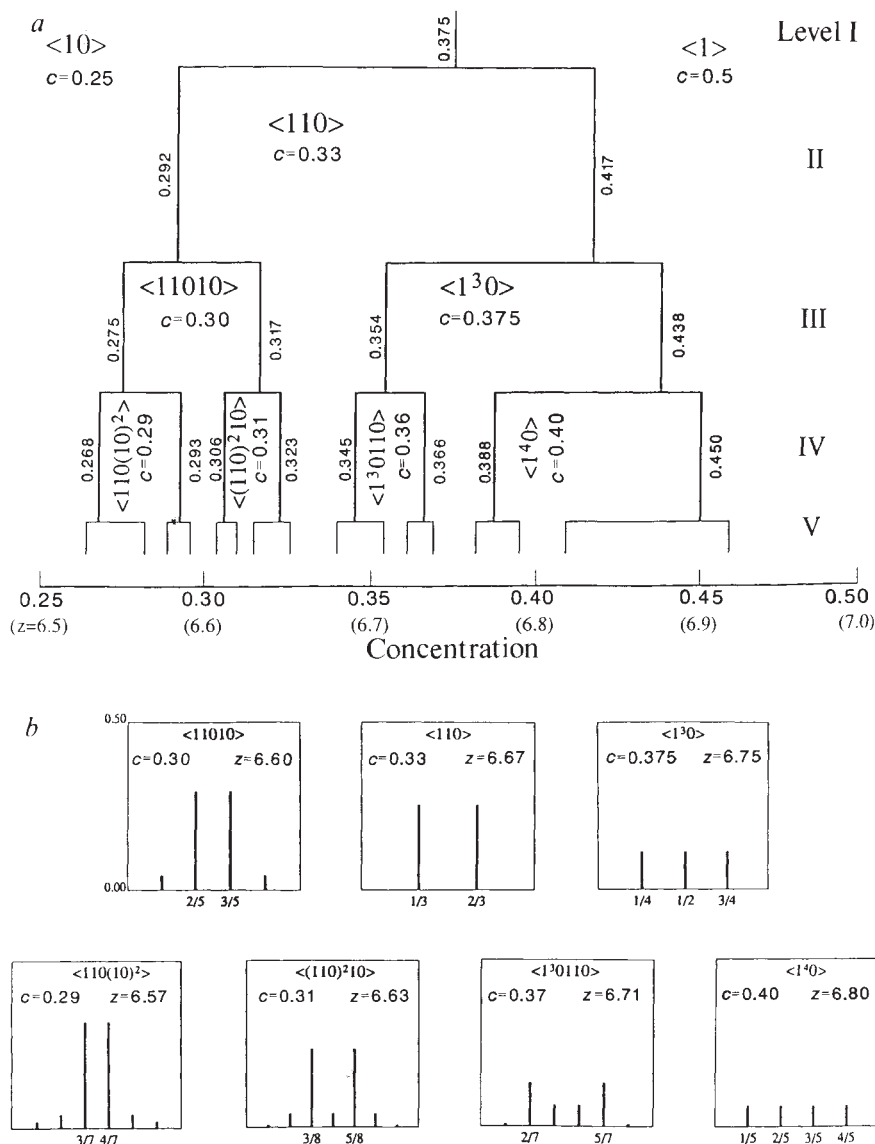


FIG. 3 *a*, Branching algorithm for quasi-one-dimensional ordered superstructures stabilized by long-range convex interactions. Concentration scale includes both basal-plane coverage (c) and stoichiometry index (z) (in parentheses). Symbols 1 and 0 indicate filled (O-Cu-O) and empty ($\square$ -Cu- $\square$) chains, respectively. Structure formulas are enclosed in angle brackets. Thus, Ortho I and Ortho II are designated $\langle 1 \rangle$ and $\langle 10 \rangle$, respectively, the brackets denoting periodic repetition. *b*, Diffraction patterns (Fourier amplitude-squared) along $(h00)$ for superstructures encountered by scanning the structure tree of *a* at Level IV, oxygen content increasing from left to right.

the observed 'unindexed' maximum at $h \approx 0.37$; clearly, it belongs to the $\langle (110)^2 10 \rangle$ structure with maximum intensity at $h = 3/8 = 0.375$. The model also accounts successfully for cell-tripling, -quadrupling and -quintupling structures observed by Reyes-Gasga *et al.*² in atomic-resolution electron microscopy.

Although neither the theoretical branching temperatures nor the temperatures at which oxygen configurations are frozen in during sample preparation are known at present, the agreement between recent experimental findings and our theoretical model is really quite striking. We are thus tempted to relate the plateau structure of the T_c versus concentration curve to the existence of a theoretically infinite set of branching phases, the T_c plateaus corresponding to states of quasi-one-dimensional order frozen in at a certain 'generation' level. The number of plateaus and their range of oxygen content should depend critically on sample preparation. Recent experimental and theoretical work support these ideas. For example, Farneth *et al.*¹⁷ report that the T_c plateau for low-temperature vacuum-annealed samples of $\text{YBa}_2\text{Cu}_3\text{O}_x$ is sharply defined and entirely located at temperatures higher than the monotonically decaying T_c versus oxygen-content curve for samples quenched from high temperatures. Lambin¹⁸ has used a tight-binding model to show that the hole concentration for well ordered Ortho II and Ortho III phases should be higher than those for the corresponding disordered compounds. It is clear that chain formation mini-

mizes the fraction of incorrectly coordinated copper ions in the basal plane. Hence, chain ordering can be regarded as a form of doping in this compound. $\square$

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A novel thin-film electrochemical device for energy conversion

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ENVIRONMENTAL concerns have led to renewed interest in fuel cells, in which gases such as hydrogen and oxygen are combined electrochemically such that the exothermicity is converted directly to electrical energy and the only reaction product is water. Conventional fuel cells are also more fuel-efficient than heat engines, but for technological reasons they are far too costly for widespread commercial use. Here I report the observation of gas $\rightarrow$ electrical energy conversion processes occurring within very thin films. I observe voltages as large as 1 volt between platinum electrodes separated by thin (<500 nm) gas-permeable, ionically conducting membranes, when one electrode is exposed to, for example, a mixture of air and hydrogen at room temperature. These observations contrast with the behaviour of conventional fuel cells, which cannot operate if the gases are mixed, and could radically simplify fuel-cell design. On the basis of these results, I suggest a simple manufacturing process by which small fuel cells could be made available at low cost.

The prototypical device (see Fig. 1) comprises a sub-micrometre-thick gas-permeable, ionically conducting membrane that is deposited on a platinized impermeable substrate (the 'inner electrode'). A second electrode, of platinum for example, is deposited on the outer surface of the membrane ('outer electrode'), and is porous enough to allow gases to pass into the pinhole-free membrane. I used hydrated aluminium oxide, pseudoboehmite (pb), as a prototypical membrane. It is a poorly crystalline, low-density form of boehmite (γ - AlOOH) with composition $\text{Al}_2\text{O}_3 \cdot 2.1\text{H}_2\text{O}$ (refs 1-4). This material normally forms on a clean aluminium surface on exposure to boiling water. Here aluminium was r.f.-sputtered onto a platinized quartz substrate (previously d.c.-sputtered with platinum) to give an aluminium film thickness of ≤ 50 nm. The assembly was immersed in boiling water until all of the aluminium had been converted to pseudoboehmite, as indicated by clarification and reappearance of the covered platinum-electrode pattern. This process was repeated to cover any pinholes developed in the lower pb layer. Finally, a thin layer of platinum was deposited on the top of the last layer of pb and the device tested in various gas mixtures at room temperature. For comparative purposes, multiple devices with different electrode materials were also made on the same substrate using the same membrane.

In hydrogen + air mixtures, the Pt/Pt device gave voltages of ≥ 950 mV, independent of the ratio of hydrogen to air for hydrogen $>50\%$. Both the polarity and the magnitude of the voltage were unexpected. The covered inner electrode was positive and the device gave open-circuit voltages comparable to those observed⁵ in conventional hydrogen/oxygen fuel cells at room

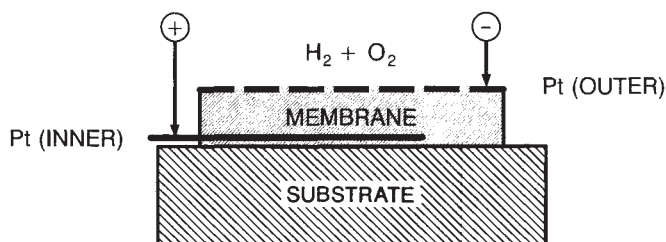


FIG. 1 Schematic cross-section of the device.

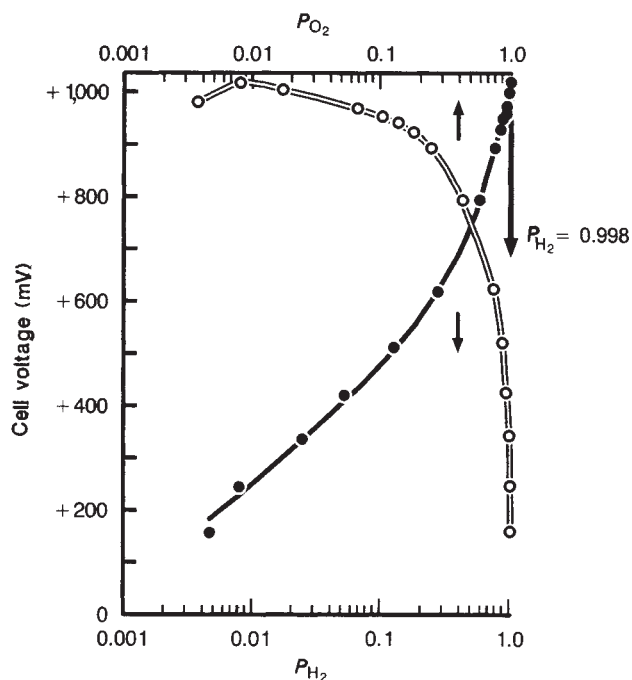


FIG. 2 Dependence of the Pt/pseudoboehmite/Pt device voltage ($\text{Pt}_{\text{inner}} - \text{Pt}_{\text{outer}}$) on the partial pressure of hydrogen (●) and oxygen (○) when the gases are mixed.

temperature and pressure (0.98–1.10 V), in which the hydrogen fuel and oxygen must be separated and purified to give such high voltages. Also, the inner pseudoboehmite-covered platinum electrode remained the oxygen (or positive) electrode over a very wide range of $\text{H}_2 + \text{O}_2$ mixtures (Fig. 2), which is remarkable. The polarity of the cell could be changed in $\text{H}_2 + \text{O}_2$ mixtures only when the outer platinum catalyst was changed to a nickel catalyst. With this Ni/Pt configuration, I measured voltages of -400 ± 20 mV for all gas mixtures in the range 10% $\text{H}_2 + 90\%$ O_2 to 90% $\text{H}_2 + 10\%$ O_2 . In cells with a nickel inner electrode and an outer electrode of platinum (Pt/Ni), the voltages were positive and more dependent on the composition of the gas mixture; in 90% $\text{O}_2 + 10\%$ H_2 , voltages of only +200 mV were observed, whereas in 90% $\text{H}_2 + 10\%$ O_2 , the voltage was +600 mV. The results are summarized in Table 1. The cell-voltage ranges shown in Table 1 for Pd/Pt and Pd/Pd were distorted by hydrogen absorption by palladium but nonetheless remained positive. The range of $\text{H}_2:\text{O}_2$ for the data shown in Table 1 was at least 10:1 to 1:10 but for the Pt/Pt cell in $\text{H}_2 + \text{O}_2$ the range was 100:1 to 10:1. I also found that the cell polarity could be reversed by changing the fuel to methanol vapour: addition of methanol vapour to Pt/Pt devices immersed in pure oxygen at atmospheric pressure gave voltages of $-620 \text{ mV} \pm 20 \text{ mV}$.

Useful current could be drawn from the cells, especially from a Pt/Pt device, in mixtures of hydrogen and oxygen or air. Figure 3 shows the short-term performance of early versions of the device. Present devices have voltage declines of only 5 mV per hour. Cell polarization characteristics were semilogarithmic in the range 200–300 mV per decade of current from 0.1 to 3.0 mA cm^{-2} . A mixture of fuel and oxygen is necessary for sustained power delivery—no voltage (to ± 1 mV) was generated in pure fuel or pure oxygen. Although the specific power levels so far seem to be low, 1–5 mW cm^{-2} , the thinness of the device is of practical importance for a lightweight power source. Built on a 125- μm -thick Kapton substrate, power densities greater

than 100 W kg^{-1} are achievable at present. Because the device is thin enough to conform to a roughened surface, power density levels of 1 kW kg^{-1} should be relatively easy to achieve. This exceeds, by an order of magnitude, present goals for electric-vehicle power sources. Cells have given sustained power for several days in a closed vessel on a single charge of gas mixture. The side reaction of direct $\text{H}_2 + \text{O}_2$ combination was reduced to a rate equivalent to 0.25 mA cm^{-2} by operating at high humidification and low total pressure. Under these conditions, coulombic (or chemical) conversion efficiencies for gas $\rightarrow$ electrical energy conversion are at present up to 50% for Pt/Pt devices and much higher for Ni/Pt.

Results similar to those reported in Table 1 were obtained using the acid polymer Nafion. In practice, this material is much easier to use than pb because it can be solution-cast to form thin films ($1\text{--}10 \mu\text{m}$) (ref. 6). Using this as the membrane material, preceded and followed by chemical deposition of metal catalysts, a continuous process can be envisaged in which the component films are sequentially built onto both sides of a moving flexible carrier (web). Because the electrodes do not need separated gas supplies and are both activated from one side, the final step would be rolling the processed web into an open spiral with a corrugated current collector to contact the outside 'negative' electrode surface and allow axial gas flow. A compact device could therefore be made quite simply and thus cheaply because there would be no need for the complex containment engineering associated with the requirement of conventional fuel cells for separated, well sealed and pressure-balanced containment of both gases and their liquid electrolyte. The use of solid electrolytes in conventional fuel cells has brought several engineering advantages, but heating is needed to raise the temperature to operating levels, which can be as high as $1,000^\circ\text{C}$ —this means a slow 'turn on' and the introduction of thermal-cycle stresses. The present device also uses a solid electrolyte, but because of its thinness it operates at room temperature which allows a rapid (millisecond) response to power demand. These observations with $\text{H}_2 + \text{O}_2$ mixtures cannot be explained satisfactorily in terms of known electrochemical mechanisms. According to the well-known Nernst relationship, the dependence of the electrode potentials on the logarithm of the gas partial pressure should be $\sim 30 \text{ mV}$ per decade. I did not observe this in a change of over two orders of magnitude in the partial pressure of O_2 in H_2 for the Pt/Pt device (Fig. 2). Also the highest voltages observed imply, using the Nernst relation, pure oxygen at the

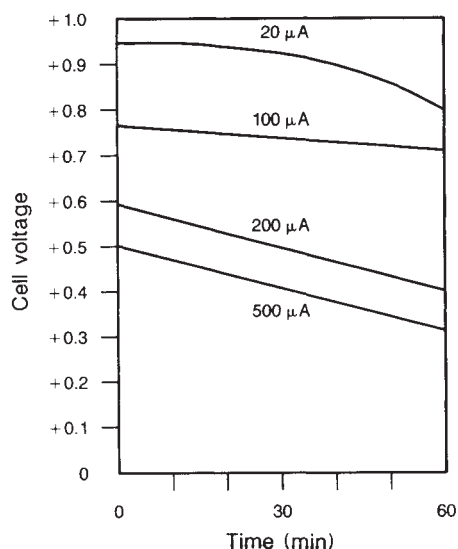


FIG. 3 Short-term performance of a Pt/pseudoboehmite/Pt power source in a 50% hydrogen/air mixture at room temperature. Area is 0.2 cm^2 .

TABLE 1 Device voltages for different catalysts using a pseudoboehmite membrane

Outer electrode material	Inner electrode material	Open-circuit device voltage (inner-outer) (mV)	Gas mixture	
			Reactant gas at outer electrode	Reactant gas at inner electrode
Pt	Pt	$+970 \pm 20$	H_2	$+$ O_2
Pd	Pt	$+540$ to $+740$	H_2	$+$ O_2
Pd	Pd	$+300$ to $+450$	H_2	$+$ O_2
Ni	Pt	-400 ± 20	O_2	$+$ H_2
Pt	Ni	$+200^*$ to $+600$	H_2	$+$ O_2
Ni	Ni	-15^* to -50	O_2	$+$ H_2
Pt	Pt	$+120 \pm 20$	CH_4	$+$ O_2
Pt	Pt	-620 ± 20	O_2	$+$ CH_3OH

* O_2 -rich.

inner platinum electrode and pure hydrogen at the outer electrode⁵. Although gettering, or rapid removal of oxygen by combination with hydrogen, at the outer electrode could increase the effective hydrogen activity at the outer electrode, the high positive potential of the pb-covered inner platinum electrode in 99% $\text{H}_2 + 1\%$ O_2 (99.9% $\text{H}_2 + 0.1\%$ O_2 with Nafion) gas mixtures is unexpected, especially when, in a separate pressure-monitoring experiment with no outer platinum electrode, direct chemical combination of H_2 and O_2 on pb-covered platinum occurred, indicating that both hydrogen and oxygen permeate pb.

The mechanism is not thermoelectric because a temperature difference between the outer and inner electrodes of $>1,000^\circ\text{C}$ would be needed to generate 1 V. This temperature gradient could not be sustained by a 500-nm-thick film. Furthermore, a cell with an outer platinum electrode made thick enough to prevent gas permeation gave no voltage to $\pm 1 \text{ mV}$.

The similarity of observations for cells using the very different membranes, pb and Nafion, indicate that selective gas transport by the membrane is also probably not involved in $\text{H}_2 + \text{O}_2$ mixtures, especially in view of the strong dependence of cell polarity on the metals used and their sequence. This suggests that different electrochemical kinetics might establish the polarities observed, perhaps by also changing the local gas ratio, more or less depending on the gettering rates on the different metals. In aqueous solutions, the fastest rates are indeed for hydrogen oxidation on platinum followed by hydrogen oxidation on nickel, then oxygen reduction on platinum or nickel⁷. This model is, however, not comprehensive enough to explain all the observations, especially the high positive potentials of the membrane-covered platinum electrode at very low oxygen pressures in Pt/Pt devices (Fig. 2).

Regardless of the precise mechanism involved, the facility with which the phenomena can be reproduced with a variety of different membrane materials should lead to rapid duplication of these results and eventually to a broad range of applications from low-cost, small, lightweight fuel cells as replacements for high-use batteries to new applications in information processing (which was the original objective of this work). □

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Oxygen isotope evidence for a stronger winter monsoon current during the last glaciation

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A VARIETY of palaeoclimate data from the northern Indian Ocean has shown that the Asian summer monsoon was weaker during the Last Glacial Maximum (~18,000 years ago) than it is today^{1–5}. It has also been inferred from these data that the winter monsoon was stronger at that time⁵. Here we present evidence that the transport of low-salinity water from the Bay of Bengal to the Arabian Sea, caused by the winter monsoon⁶ was higher during the Last Glacial Maximum. This inference is based on a negative excursion, up to about 1‰ in magnitude, in the oxygen isotope ratios of three planktonic foraminiferal species, detected in a sediment core from the Arabian Sea by high-resolution (1–2 cm) sampling. Our results place an upper limit of about 4,000 years on the length of time during which the winter monsoon was stronger than today.

A plot of the ¹⁴C age as a function of depth is shown in Fig. 1 for two cores, SK-20-185 (10° N, 71°50' E, 2,523 m water depth) and SK-20-186 (0°2' S, 68°30' E, 3,564 m); the core locations along with the winter salinity pattern is shown in Fig. 2. Core-top ages for both the cores are ~4 kyr. This could be due to benthic mixing or due to the loss of a few centimetres of the core top during retrieval of the cores.

For $\delta^{18}\text{O}$ analyses ($[(^{18}\text{O}/^{16}\text{O})_{\text{sample}}/(^{18}\text{O}/^{16}\text{O})_{\text{standard}} - 1] \times 10^3$, PDB belemnite standard), 30–40 individual shells (size fraction: 250–400 μm) were reacted with 100% H_3PO_4 at 50 °C and water vapour removed thoroughly from the evolved CO_2 before analysing its $\delta^{18}\text{O}$ in a VG Micromass 602 D mass spectrometer. The overall analytical precision was $\pm 0.1\%$ on standard carbonates (Z-Carrara and Marble V), and for foraminiferal samples, the mean difference between 100 duplicate analyses was 0.15%. Details are reported elsewhere⁷.

Figure 3 shows $\delta^{18}\text{O}$ values of four species of foraminifera *Globigerinoides sacculifer*, *Orbulina universa*, *Globigerinoides ruber* and *Globorotalia menardii* in core SK-20-185 from depths of 2 to 48 cm and ¹⁴C ages. Table 1 summarizes the isotope data.

The most notable feature of Fig. 3 (enclosed between the two vertical dashed lines) is the negative excursion of $\delta^{18}\text{O}$ during the Last Glacial Maximum (LGM) (18 ± 1.5 kyr ago, ref. 3) lasting apparently for 4 kyr (16–20 kyr). Although all four species show this, the magnitudes are different: ~1‰ in *G. sacculifer* and *O. universa*, ~0.6‰ in *G. ruber* and ~0.3‰ in *G. menardii* (the coarse fraction (>150 μm), mainly foraminiferal shells, also reached a minimum of 4‰ during this period which probably indicates decreased productivity, Fig. 3f). Although there is some uncertainty^{8–10} regarding the exact depth at which these species live, it is generally believed that the former three record surface-ocean conditions whereas the latter (*G. menardii*) is deeper dwelling and does not record variations in the temperature and salinity of the euphotic zone⁵. Because the magnitude of the pulse is much less in *G. menardii*, we infer that the mechanism responsible for this pulse was predominantly of surface origin. Also, no difference in $\delta^{18}\text{O}$ values between peak and background levels was found in another deeper-dwelling species *Pulleniatina obliquiloculata* (Fig. 3e). We confirmed the negative pulse in $\delta^{18}\text{O}$ by repeated analyses of the four species extracted from multiple aliquots of the sediment. Thus, there was a period of up to ~4 kyr during the LGM, when surface-dwelling foraminifera in this region secreted isotopically lighter CaCO_3 shells.

Benthic mixing of sediments usually precludes the possibility of obtaining high-resolution information, especially from cores with low sedimentation rates. A signal can be detected, however, if the mixing scale length is low or if the amplitude of the signal is large enough that the effects of bioturbation do not wipe it out. We believe the latter to be the case for our core, as the signal is detected even without the use of any unmixing procedure. Such unmixing will only sharpen the pulse¹¹, apart from introducing some spurious noise¹².

We assume that bioturbation was not significant at the location of core SK-20-185 during the LGM, because at this depth, the sediments are layered with black organic-rich bands. The bottom water was probably oxygen-poor¹³, resulting in a better preservation of organic matter. In such conditions the occurrence of burrowing benthic organisms is less likely. Recent evidence¹³ shows that depleted oxygen conditions prevailed during glaciation in the deep water of the northeastern Indian Ocean. This is based on the absence of *Cibicides* in sediment cores deeper than 2,600 m, which usually lives in well oxygenated sea water.

Modern hydrographic conditions at the core (SK-185) location indicate a decrease in the surface temperature of 1–2 °C during the summer (south-west) monsoon, probably caused by the deepening of the mixed layer, induced by the monsoon winds¹⁴. The winter (north-east) monsoon current transports low-salinity water from the Bay of Bengal to the eastern Arabian sea following a route south of Sri Lanka⁶ (arrows in Fig. 2), decreasing the surface salinity at the core location by ~1‰. If during the LGM, the summer monsoon weakened^{1–5}, the deepening of the mixed layer would vanish, resulting in a relative warming of 1–2 °C and a consequent decrease in the $\delta^{18}\text{O}$ of planktonic foraminifera of up to ~0.5‰. Furthermore, if the winter monsoon (atmospheric) circulation was stronger, increased precipitation in the south-east coast of India and increased run-off from the southern rivers would result in a lower salinity in the southwestern Bay of Bengal. (Today, a large portion of the annual rainfall is received during the north-east monsoon in Tamil Nadu and eastern Andhra Pradesh.) This, coupled with the intensification of the north-east monsoon

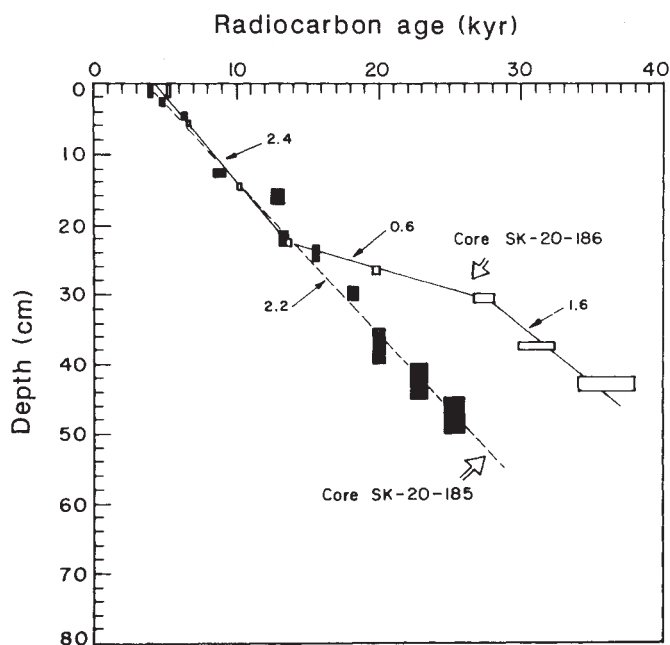


FIG. 1 Plot of ¹⁴C age as a function of depth in cores SK-20-185 and SK-20-186. Sedimentation rates (cm kyr^{-1}) are indicated. Note the breaks in sedimentation in SK-20-186. A half-life of $5,730 \pm 40$ yr was used. Experimental details are reported elsewhere¹⁸.

TABLE 1 Oxygen isotope data for cores

Depth (cm)	SK-20-185					SK-20-186	
	1	$\delta^{18}\text{O}$ (‰)	2	3	4	5	6
2-3	-1.34	-1.49	-1.87	-0.81	-0.94	0-2	-1.37
4-5	-1.72	-1.61	-2.24	-0.82	—	2-3	-1.39
6-7	-1.70	-1.44	-2.23	-1.02	-0.96	11-12	-1.10
8-9	-1.41	-1.38	-1.90	-0.74	-1.04	13-14	-0.85
10-11	-1.17	-0.69	-1.89	-0.21	-0.58	15-16	-0.51
12-13	-0.60	-0.63	-1.17	-0.15	—	17-18	-0.49
14-15	-0.94	-0.52	-1.45	-0.52	-0.11	19-20	-0.57
16-17	-0.46	0.01	-1.37	-0.04	-0.04	21-22	-0.47
17-19	-0.16	0.08	-0.61	0.64	—	24-25	-0.32
19-21	-0.16	-0.12	-1.21	0.15	0.12	26-27	0.00
21-23	-0.09	0.38	-0.72	0.51	0.55	28-29	0.13
23-25	0.08	0.38	-0.48	0.78	0.82	30-31	-0.30
25-27	0.23	0.43	-0.21	0.87	1.03	32-33	-0.31
27-29	-0.14	0.23	-0.42	0.59	0.85		
29-31	-0.30	0.31	-0.53	0.87	—	42-44	-0.37
31-33	-0.97	0.05	-0.75	0.40	1.13	46-48	-0.46
33-35	-0.88	-0.69	-0.67	0.65	1.13	52-54	-0.83
35-40	0.34	0.65	-0.28	0.80	0.95	56-58	-0.70
40-45	0.26	0.20	-0.42	0.57	—	62-67	-0.68
45-50	0.26	0.22	-0.63	0.79	0.95		

1 and 6, *G. sacculifer*; 2, *O. universa*; 3, *G. ruber*; 4, *G. menardii*; 5, *P. obliquiloculata*. Size 250–400 μm except for *O. universa* (400–500 μm).

(ocean) current, would have transported an increased amount of lower-salinity water to the core location. If the salinity was thus lowered by $\sim 2\%$, then the $\delta^{18}\text{O}$ of the foraminifera would have reduced by $\sim 0.6\%$. (A 1‰ drop in salinity reduces $\delta^{18}\text{O}$ by $\sim 0.3\%$ in this region⁹.) Thus a total reduction of $\sim 1\%$ in the $\delta^{18}\text{O}$ would result because of these two factors. *G. menardii*, being deeper dwelling, records only the lack of deepening of the mixed layer ($\sim 0.3\%$) whereas the other species show a 1‰ decrease. *G. ruber*, though surface dwelling, shows a reduced effect ($\sim 0.6\%$) possibly because its abundance peaks in summer and fall whereas *O. universa* and *G. sacculifer* have more or less uniform abundances throughout the year¹⁵.

Earlier work³ in the northern Indian Ocean has revealed that both the Bay of Bengal and the Arabian Sea were more saline during the LGM than in the Holocene. But that study did not include a core from the western Bay of Bengal, which is under the influence of east-bound peninsular rivers. Although the

winter monsoon is generally considered to be dry and continental in nature, it picks up moisture from the Bay of Bengal and precipitates over the eastern part of the peninsular India, occasionally giving rise to floods. Even today, a reduction in salinity in the western Bay of Bengal is seen¹⁴ during winter. Another way to explain the lower salinity would be to consider that the Bay of Bengal was less saline during the LGM⁹ relative to the Arabian Sea. As the local ocean circulation was dominated by the winter monsoon during the LGM, the core location (SK-185) received low-salinity water by westward advection.

We also performed $\delta^{18}\text{O}$ analyses, with similar high resolution, on samples of *G. sacculifer* from another core taken in the equatorial region (SK-20-186, Table 1, Fig. 2). The negative excursion of $\delta^{18}\text{O}$ during the LGM is absent in this core, probably because then, as today, the winter monsoon current did not reach this location⁶. This conclusion is not certain, however, because there was a reduction in the sedimentation rate (from

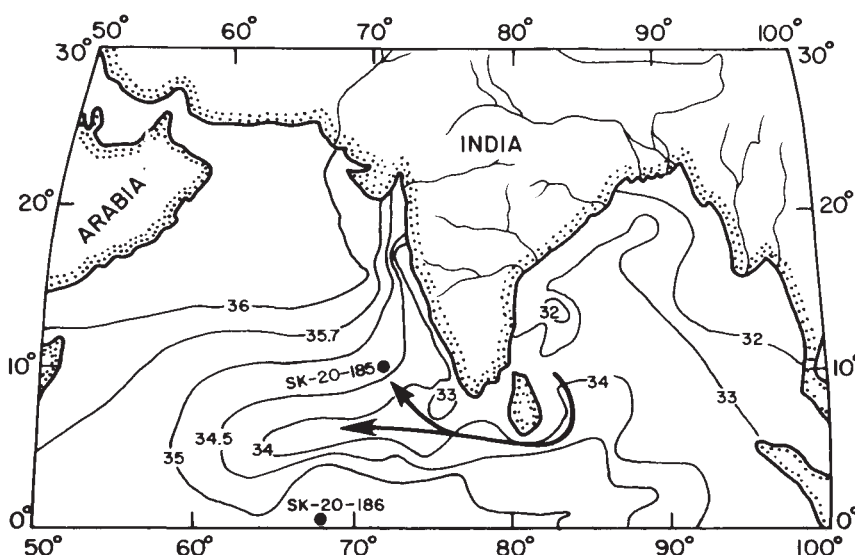


FIG. 2 Map of northern Indian Ocean showing the core locations. The north-east monsoon current that brings low-salinity water from Bay of Bengal to the eastern Arabian Sea during the winter monsoon is shown by thick arrows. Salinity contour during winter are also shown.

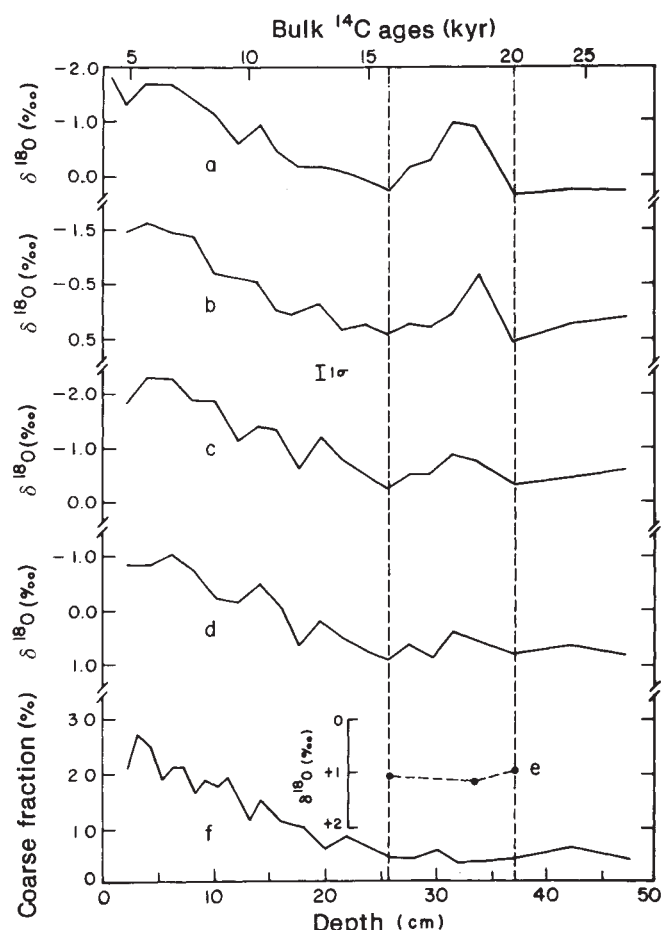


FIG. 3 $\delta^{18}\text{O}$ values as a function of depth in core SK-20-185 for a, *G. sacculifer*; b, *O. universa*; c, *G. ruber*; d, *G. menardii*. f, Percentage coarse fraction is also shown. Radiocarbon dates of bulk sediment are shown on the top. The annotations of the ^{14}C -age axis indicate integer values for clarity and the positions of the tick marks were estimated using actual ages. e, $\delta^{18}\text{O}$ values of *P. obliquiloculata* are shown for comparison.

2.4 to 0.6 cm kyr⁻¹, Fig. 1) during the LGM, which may indicate a hiatus during this period.

Our results indicate that the phase during which the winter monsoon was stronger lasted for up to 4 kyr during the LGM. This was possibly caused by the build-up of glacier ice over Himalaya and Tibetan plateau, which enhanced the land-to-sea temperature contrast in winter and reduced it during summer⁵. This would result in a weaker summer monsoon⁵ and in a strengthening of the winter monsoon, as indicated by our study. The earlier studies that indicate the strengthening of the winter monsoon during the LGM are based on land-derived material. Van Campo¹ *et al.* found a large influx of Asian pollen in the sediments of the Arabian Sea. Kolla and Biscaye¹⁶ reported increased amounts of quartz transported from Asia by strong north-east winds into the Arabian Sea, and evidence for the intensification of sand-dune building activity in the Thar desert of Rajasthan has also been found¹⁷. □

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Evidence from chromium abundances in mantle rocks for extraction of picrite and komatiite melts

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ONE of the more important aspects of understanding the geochemical differentiation of the Earth is an accurate knowledge of the compositions of magmas produced by direct melting of the upper mantle, which are called primary magmas¹. It is clear that a variety of primary magma compositions can develop within the mantle, depending on the source composition, the presence of volatiles, the extent of melting and the pressure of melt segregation^{1–4}. Proposed primary magma compositions span a wide composition range, from basalts with ~8–11% MgO, through picrites with ~11–20% MgO, to komatiites with ~20–28% MgO; there is no agreement as to which is the dominant type of primary magma. We suggest here that the abundances of chromium in samples of the Earth's upper mantle indicate that, of the wide variety of possible primary liquids produced in experimental studies of mantle melting, the dominant type of primary liquid actually extracted from the mantle has been picritic to komatiitic, with $\geq 15\%$ MgO.

The abundances of major and minor elements in upper-mantle peridotites from numerous terrains (xenoliths, alpine peridotites, abyssal peridotites and ophiolites) have systematic variations that are thought to result from varying degrees of partial melting and melt extraction^{1,5–8}. The linear relationships between various components (such as SiO₂, TiO₂, Al₂O₃, CaO, Na₂O, Ni, Co, Sc, Cr and Yb) and MgO have often been used to define melt extraction trends (METs) such as those shown schematically in Fig. 1. Previous studies of peridotites^{7–16} show that plots of SiO₂, TiO₂, Al₂O₃, CaO, Na₂O, Sc and Yb against MgO have negative slopes. A negative slope indicates that the bulk distribution coefficient¹⁷ is less than unity during melting, consistent with the higher abundances of these components in basaltic liquids than in mantle peridotites. These studies have also shown that the plot of Ni against MgO (Fig. 2a) has a positive slope, consistent with a high (>1, probably 3–7) bulk distribution coefficient for Ni during mantle melting. When the MET is horizontal (Fig. 1), the element has a bulk distribution coefficient of unity and the primary magma has the same concentration of that element as the mantle.

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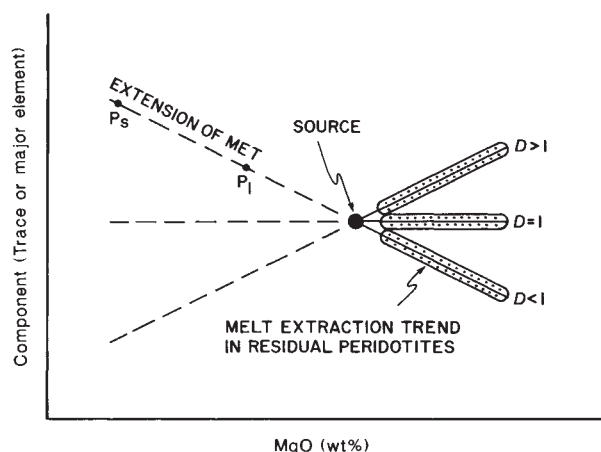


FIG. 1 Melt extraction trends for three components with different bulk-rock distribution coefficients, D . The three elongate fields with higher MgO contents than the source represent analyses of residual mantle rocks for which METs are defined as the solid lines. For each D , the average melt extracted from the mantle lies along the linear extension (dashed line) of the MET. P_s is the primary magma produced by a small amount of melting and P_l is the primary magma produced by a large amount of melting.

When composition variations within a suite of peridotites are dominated by partial-melting processes, the MET can be used to constrain the average primary magma composition extracted from the suite of peridotites, because it must lie somewhere along the extension of the MET. The exact position of the average primary magma along this extension can be constrained using a well-known distribution coefficient; the Fe/Mg partitioning between olivine and liquid¹⁸ has generally been used.

For most elements, the bulk distribution coefficients are similar during melting and crystallization (at least when olivine, pyroxene and spinel dominate) and the MET for peridotites is collinear with basalts, picrites and komatiites. As an example, the Ni-MgO MET for peridotites is essentially collinear with basalts, picrites and komatiites (Fig. 2a) and is, therefore, consistent with the extraction of almost any of these magma types. For the purposes of defining the average composition of primary melt extracted from a suite of peridotites, the best element would be one for which the bulk distribution coefficient during melting is much different from that during crystallization. In such a case, there would be a large angle between the MET and the liquid line of descent produced during crystallization.

Studies of mafic and ultramafic liquids show that Cr and MgO abundances decrease with the crystallization of olivine, pyroxenes and chrome spinel (see Fig. 2b, where MgO < 20%). Cumulate ultramafic rocks have high Cr (generally >1,500 p.p.m.) and MgO (>18%) contents¹⁹; the bulk distribution coefficient for Cr during the crystallization of cumulate ultramafic rocks is ~3–10 for mixtures of olivine, orthopyroxene and/or clinopyroxene with 1–2% chrome spinel²⁰. In cumulates with abundant chrome spinel, the bulk distribution coefficient for Cr can be >250 (ref. 20). There is a general belief that Cr behaves as a compatible element during mantle melting in which Cr is preferentially retained in the residual mantle, but previous studies of peridotites^{1,7,21} have suggested that the bulk distribution coefficient for Cr is ~1 during melting of the mantle, compared to the high values (3–10) during crystallization.

The Cr and MgO abundances of mantle samples are available for xenoliths from alkali basalts, alpine peridotites and other peridotite suites^{1,5–16,21–27}. Here we have not used residual-mantle samples from ocean basins because they have undergone substantial hydrothermal alteration and serpentinization, which often results in an increase in MgO (ref. 28) and cannot be used reliably for defining melt extraction trends. We have also excluded peridotites with any significant amount of plagioclase or amphibole because studies⁶ have indicated that these samples have generally been metasomatized or impregnated with basaltic liquids.

On a plot of Cr against MgO, the MET established for most of these mantle samples is essentially horizontal (Fig. 3a–c). In each of these suites, the Cr contents for the samples with the highest MgO (most depleted) are about the same (slightly higher

to slightly lower) as the samples with the lowest MgO (least depleted). There is generally more scatter in the Cr–MgO data within a given suite (see Fig. 3a–c) than for compatible elements such as Ni or Co plotted against MgO (refs 7, 9, 21); this scatter is larger than analytical uncertainties. It seems that the most significant factor in this scatter is the difficulty in obtaining a representative mantle sample for analysis; Cr is distributed heterogeneously within mantle samples and many samples are quite small. This problem with heterogeneous sampling is particularly important where the amount of material analysed is small (for example, neutron activation analysis often involves samples weighing <150 mg). Mantle heterogeneity and variable oxidation states of the mantle during melting also contribute to this scatter. In spite of the scatter, it is significant that most depleted peridotites have approximately the same Cr abundances as the least depleted samples and that the MET is horizontal.

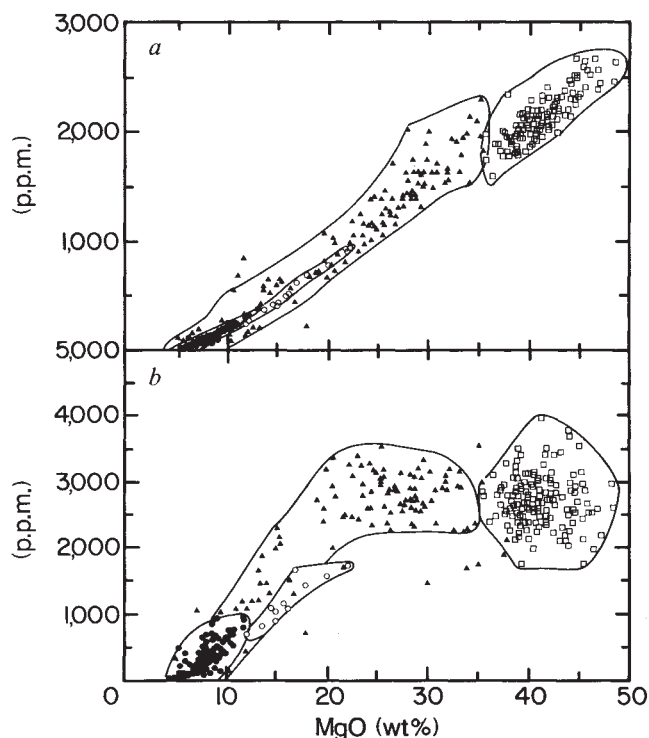


FIG. 2 Variation diagrams of a, bulk-rock Ni with MgO and b, Cr with MgO for mid-ocean-ridge basalts (MORBs (●)), peridotites (□), picrites (○) and komatiites and associated mafic rocks (▲). Sources of data are available on request.

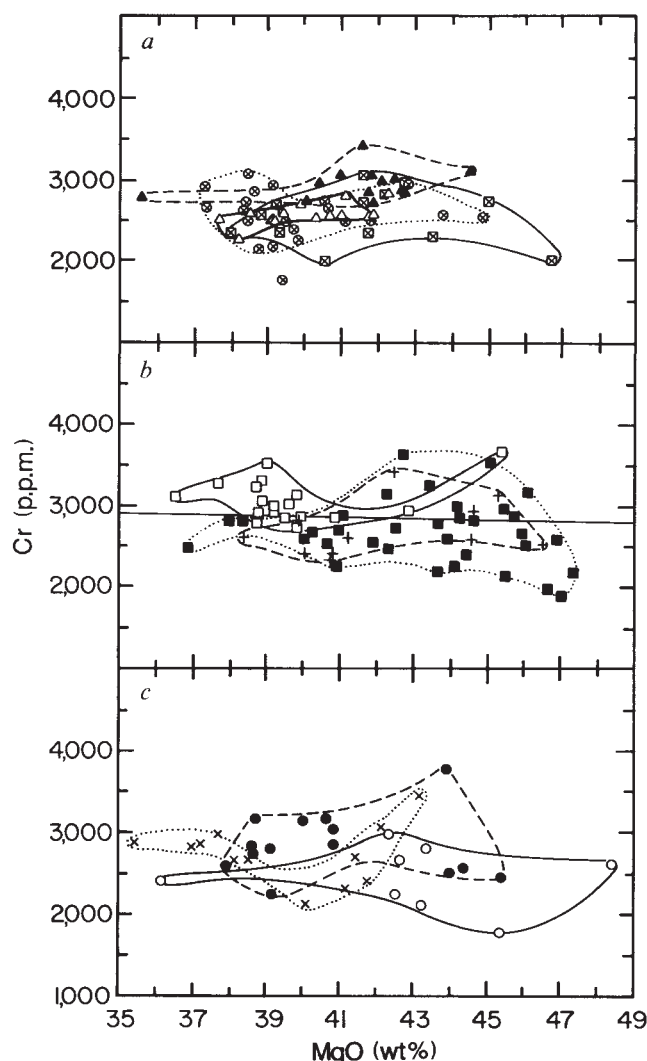


FIG. 3 Variation diagrams of bulk-rock Cr with MgO for *a*, alpine peridotites and *b*, *c*, peridotite xenoliths from alkali basalts. The nearly horizontal line in *b* represents a regression analysis of 300 ultramafic xenoliths from alkali basalts³⁶. In *a*: ▲, Tinaquillo, Venezuela; △, Zabargad, Red Sea; □, Ronda, Spain; ⊗, Western Alps. In *b*: +, Yukon, Canada; ■, Southeast Australia; □, Kilbourne Hole, USA. In *c*: ●, Hungary; ○, Dreiser Weiher, FRG; ×, Mongolia.

These horizontal METs indicate that the bulk distribution coefficient for Cr during melting of the mantle is ~ 1 . These results indicate that the average primary magmas extracted from the mantle had $\sim 2,000$ – $3,000$ p.p.m. Cr, much higher than basalts with 8–11% MgO, which have 200–600 p.p.m. Cr (Fig. 2*b*). Komatiites or picrites with $>15\%$ MgO have $\sim 1,800$ – $3,000$ p.p.m. Cr, indicating that the melts extracted from these mantle samples were picritic to komatiitic (Fig. 2*b*). Picritic liquids seem to be produced by separation of melts from the mantle at 20–35 kbar (refs 3, 4, 29) and komatiitic liquids seem to be separated from the mantle at 40–150 kbar (refs 30–33), where the mantle solidus and liquidus are converging or have converged^{31–33}.

Detailed studies of mantle peridotites indicate that their history has been complex^{7,34}. For this reason and because of the aforementioned scatter in the data, precise estimates of the compositions of individual primary magmas are difficult to constrain; so far, we can give only broad categories of primary magmas (such as komatiite and picrite with $\geq 15\%$ MgO).

The advantage in using Cr–MgO METs to define the general

category of primary magmas extracted from a peridotite suite is that the bulk distribution coefficient for Cr is ~ 1 during mantle melting, which means that estimates of the Cr abundances in primary magmas are essentially independent of assumptions regarding other distribution coefficients or the extent of melting. These results indicate that the dominant phase of melting mantle peridotites involved the extraction of picritic–komatiitic liquids with $\geq 15\%$ MgO; these primary magmas must crystallize a moderate amount (10–35%) of olivine, pyroxene and chrome spinel in order to form the common basalt types with ~ 8 – 11% MgO that are commonly erupted near the Earth's surface. The details of how komatiites and picrites evolve to common basalts with 8–11% MgO are not well constrained at present because of limited experimental data and the altered states of most of these lavas. It is clear, nonetheless, that crystallization of olivine, pyroxenes and spinel in komatiites and picrites produces a liquid line of descent that leads to basalts with 8–11% MgO (ref. 35; see also Fig. 2*b*).

Komatiites and picrites seem to have been the dominant type of primary liquid extracted from the mantle, but the role of other magmas and the timing of melt extraction is uncertain. Two general models seem to satisfy the available data. One possibility is that the dominant phase of melt extraction recorded in these peridotites occurred during the Archaean ($>2,500$ Myr ago), when komatiite magmatism was common, and that it produced a horizontal Cr–MgO MET. In this model, more recent basaltic magmatism such as that at mid-ocean ridges to produce MORBs ($<11\%$ MgO and <600 p.p.m. Cr) would superimpose a series of subsidiary (sloping) trends on the horizontal trend and introduce scatter in the MET. The other possibility is that extraction of picritic and komatiitic liquids from the mantle has been, and is at present, the dominant form of mantle melting. In this model, picrites and komatiites developed in the present-day mantle would undergo substantial crystallization before eruption as basalts. It is likely that the resolution of this uncertainty will come from detailed studies of compatible and moderately incompatible elements in peridotites. □

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Resistance to positional noise in human vision

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HUMAN eyes are in constant and rapid motion even when observers try to maintain steady fixation^{1,2}. Also, the visual system has a sluggish temporal response³. In combination, these two factors would be expected to blur stimuli and reduce spatial sensitivity^{4,5}. But observers are able to detect a difference in separation of a few seconds of arc between two closely spaced parallel lines⁶. Here we report that even very large amounts of positional jitter of the line pair has minimal impact on this ability. This result is in marked contrast to the deterioration observed when targets are swept linearly across the retina⁵, but is consistent with a system that must ignore oculomotor jitter. To explain these results will require a re-evaluation of current models of position coding in human vision^{5,7,8}.

We measured the ability of observers to detect small changes in the separation of two parallel vertical lines 10 arcmin in height. The observers were shown a pair of lines for 600 ms, followed by a 100-ms dark interval and then a second pair for a further 600 ms. The observer's task was to indicate whether the second line pair appeared narrower or wider than the first. All viewing was binocular and feedback was provided for incorrect responses.

We used two line-separations because the salient cue is believed to alter as separation increases. The larger was 6 arcmin where positional differences are the cue^{5,7,9}. The smaller was 2 arcmin where, because the lines are blurred by the optics of the eye, the depth of the luminance decrement between the two lines is the cue^{7,9}. Retinal image motion produced by oculomotor instability changes direction rapidly, so the resulting superimposition of targets on the retina would be expected to cause serious

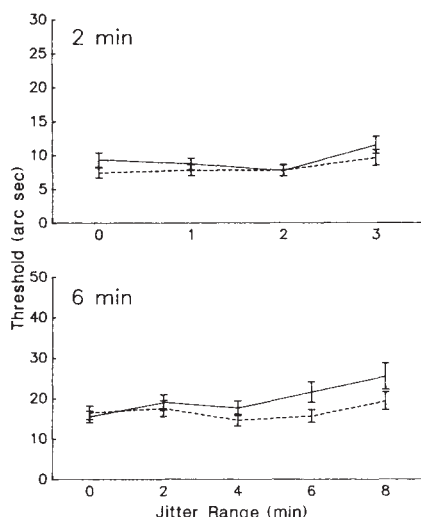


FIG. 1 Separation discrimination thresholds (75% correct) collected using standard methods⁶ are plotted as a function of the range of target jitter. Observers (DRB, dashed line; TLW, solid line) indicated whether the second pair of target lines was wider or narrower than the first pair. The 600-ms targets were intensified to 0.18 cd m^{-2} in a new randomly chosen location for 3 ms in every 30 ms. The two panels show performance with either a 2-arcmin (upper) or 6-arcmin (lower) reference separation. Performance is largely unaffected by jitter.

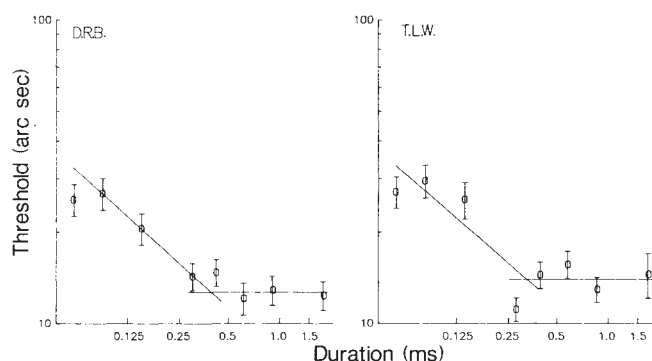


FIG. 2 Separation discrimination thresholds (75% correct) for D.R.B. and T.L.W. are plotted as a function of stimulus duration. The reference separation was 6 arcmin and all procedural details were the same as for Fig. 1. Stimulus duration was varied by altering the number of 30-ms epochs in each interval. The solid lines have been fitted by eye with the constraint that the first line has a slope of -0.5 (as performance would be expected to improve in proportion to the square root of the number of samples) and the second has a slope of zero. The point of intersection of the lines is an estimate of the temporal integration time for separation discrimination. The target lines were not jittered in location.

difficulty for judgments of small separations. But separation discrimination is usually much better for small separations^{6,7}. We exaggerated the effect of oculomotor jitter by randomly displacing the line pair to the left or to the right every 30 ms. The range of displacements was varied, but the maximum range allowed the two lines to appear in spatially overlapping ranges of locations during the interval (a range of 8 arcmin for the 6-arcmin separation, and a range of 3 arcmin for the 2-arcmin separation). Oculomotor jitter can have a s.d. as little as 0.25 arcmin when observers are carefully fixating for 400 ms (ref. 10) and as large as 1.8 arcmin for durations of 1 s (ref. 2). Within an interval, the lines had equal probability of taking any position in the jitter range. Different sets of offsets were used in each interval to eliminate meaningful cues from the location of the outermost lines in the set.

The lines were displayed on a high-resolution oscilloscope (HP1345, P4 phosphor) under computer control. The results shown in Fig. 1 are the thresholds of the authors (the main findings have been confirmed with naive observers). Performance was only minimally affected (less than a factor of two) at either separation by positional jitter that mimicked an exaggerated form of oculomotor instability.

Morgan and Benton⁵ found that linear motion sweep did cause performance to deteriorate with a 4.5-arcmin line separation. With their method, however, the time the target is presented on the fovea decreases as the stimulus velocity increases. The deterioration in performance could have been due to the shorter stimulus presentation time in the region of highest sensitivity^{6,11}. The experimental stimuli that we used were always within a few arcmin of the centre of the fovea. We also chose a longer duration of presentation than did Morgan and Benton⁵, so that our stationary stimuli would produce optimal sensitivity and thus be most likely to show adverse effects of jitter¹².

Morgan and Benton⁵ proposed that the deterioration in the performance of separation discrimination is due to the blurring of the contrast cue provided by the dip in the luminance profile between the two lines. The blurring was believed to be a consequence of the trailing line sweeping across the retinal area that had contained the gap. In the experiment described here, the lines were repeatedly displayed in random locations within a small retinal area. The contrast cue should have been reduced even more severely. But in fact performance was affected much less. This does not mean that with the small separations the

contrast cue was not used by our observers. If that cue was used, however, its extraction was performed within 30 ms. This would be surprising, given the 50–100-ms duration of the human response to temporal impulses¹³, and that the performance of separation discrimination improves with durations up to at least 300 ms (D.R.B. & T.L.W., manuscript in preparation) as shown in Fig. 2.

The results presented here additionally argue against models that propose that pattern motion is discounted by motion-deblurring or linear interpolation systems^{4,14,15}. These models are not designed to account for equally good performance with random and rapid changes of image location. Models based instead on the use of contrast and position cues^{7–9} would also need to be modified to make the degradation of the contrast cue with jitter of small separations visually ineffective. The results described above show that more adequate attention needs to be given to the temporal properties of the models. It is not clear how this resistance to positional noise could best be incorporated. But it must be included if the models are to adequately

represent the human visual system, which is superbly designed to discount the noise introduced by oculomotor instability. □

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Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma

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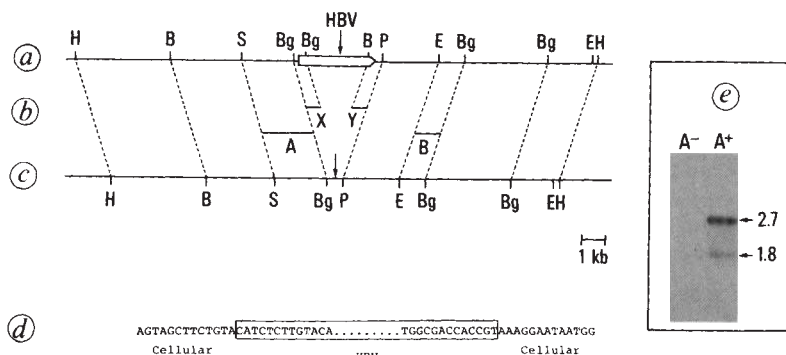
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HEPATITIS B virus (HBV) DNA frequently integrates into the genome of human primary liver cancer cells^{1–4}, but the significance of this integration in liver carcinogenesis is still unclear. Here we report the cloning of a single HBV integration site in a human hepatocellular carcinoma at an early stage of development, and of its germline counterpart. The normal locus was found to be transcribed into two polyadenylated messenger RNA species of 1.8 and 2.7 kilobases. We have isolated a complementary DNA clone from a normal adult human liver cDNA library which has an open reading frame with a coding capacity for a protein of 432 amino acids and relative molecular mass 48,536. The strong homology of the C-terminal half of the protein to the A-type cyclins of clam⁵ and *Drosophila*⁶ identifies it as a human cyclin A. The cyclin A gene has several exons, and the HBV integration

occurs within an intron. As cyclins are important in the control of cell division^{7–17}, the disruption of a cyclin A gene by viral insertion might contribute to tumorigenesis.

We have analysed a very early, well-differentiated primary hepatocellular carcinoma, which developed as a single small nodule without accompanying cirrhosis in a hepatitis B surface antigen-positive female. Southern blots of DNA prepared from non-tumour and tumour tissues were probed with HBV DNA. In non-tumour tissue, only free HBV DNA was identified, but the tumour DNA gave a unique band, corresponding to one integrated HBV genome copy per cell (data not shown). We constructed a phage library from tumour DNA, and have isolated several overlapping clones with an HBV-specific probe. The resulting restriction map of the HBV provirus and flanking cellular sequences in the tumour is shown in Fig. 1a. Figure 1b shows the subclones A, X, Y, B, free of repetitive sequences, that were derived from the phage clones. Sequencing of the provirus–host junction fragments X and Y (Fig. 1b), together with a detailed restriction analysis of the proviral insert, yielded a viral genome without major rearrangements. The viral–cellular junctions are both located in the HBV cohesive ends region (nucleotides 1,840 and 1,617 in the viral core and X open reading frames, respectively¹) (Fig. 1d). Hybridization of Southern blots made from tumour DNA with probes A and B (Fig. 1b) confirmed the genomic configuration described in Fig. 1a (data not shown). Using probe B, a 1.1-kilobase (kb) *EcoRI/BglII*

FIG. 1 a, Restriction map of the HBV integration region in the tumour. The open box represents the 3.1 kb viral insert indicating the viral (+) stand orientation. Restriction sites are: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sst*I. b, Subclones derived from the tumour genomic sequences shown in a, all free of repetitive sequences. A is a 2.2 kb *Sst*I/*Bgl*II fragment; B is a 1.1 kb *Eco*RI/*Bgl*II fragment. Fragments X (0.5 kb *Bgl*II) and Y (0.7 kb *Bam*HI/*Pst*I) contain the left and right virus–host recombination sites, respectively. c, Restriction map of the HBV integration locus in its germline configuration. The arrowhead points to the site of viral integration in the tumour; dotted lines between restriction sites in a and c denote identical restriction fragments. d, Sequence of the virus–host junctions (see text). e, Northern blot of human newborn liver RNA, probed with clone B (see b). A[–], 50 µg poly(A)[–] RNA; A⁺, 5 µg poly(A)⁺ RNA. Sizes are given in kb. METHODS. A phage library was constructed by partial digest of the tumour DNA with *Hind*III and ligation into phage λL 47 *Hind*III arms. A set of overlapping phage clones was isolated with cloned HBV as a probe. Subcloning was performed with Bluescript vectors (Stratagene). The germline allele



(Fig. 1c) was cloned from an EMBL 3 library made from placental DNA, partially digested with *Sau*3AI. Sequencing was by the dideoxy chain termination method³⁹. The cDNA was isolated from a library constructed from oligo(dT)-primed adult human liver mRNA (a gift from D. Lamy and C. de Taisne³²).

nor a polyadenylation consensus signal.

A computer search of a protein data base shows that the predicted protein contains a region of 150 amino acids (at positions 210–359 in the C-terminal half, see Fig. 2) which is typical of a cyclin box⁹, the domain of greatest conservation among all known cyclins^{4,5,7,9,12}. An alignment (Fig. 3) shows that in this region it shares 77 out of 150 amino-acid positions with the cyclin boxes of cyclin A from both *Spisula solidissima*⁴ and *Drosophila melanogaster*⁵. In another 47 positions, it diverges only by conservative substitutions from one or both. As it has only 54 out of 150 amino-acid positions in common with the cyclin boxes of cyclins B from both *S. solidissima*¹ and *man*¹², the predicted protein must be a human cyclin A. The N-terminal half of the protein does not share substantial homology with other known proteins, including other cyclins, which is a common feature of the known cyclins.

In Southern blots of restriction digests of the germline phage clones, fragment A (Fig. 1b) was positive for the cDNA probe (Fig. 1); we therefore partially sequenced fragments A and B and found sequences corresponding to the 5' and 3' ends of the cDNA, respectively. The deduced orientation of the cyclin A gene is from left to right in Fig. 1 (not shown). Hence, the cyclin A gene has several exons and, given that the cellular sequences in the viral–host junction fragments X and Y (Fig. 1b) are not found in the cDNA, the HBV integration in the tumour has occurred in an intron of the gene.

By investigating an HBV DNA integration site, we have identified a human cyclin A gene. Cyclins were first identified in cleaving eggs of marine invertebrates⁷. They are characterized by their steady accumulation throughout interphase until the G₂/M transition, followed by rapid disappearance at the onset of anaphase. They are highly conserved in evolution and have been identified in yeast, clam, starfish, sea urchin^{13,14} and *Drosophila*⁶. Recently a human cyclin B cDNA was isolated¹². The two groups of cyclins (A and B) are distinguished on the basis of their sequence and pattern of accumulation during the cell cycle^{5,15}. It has been demonstrated that both cyclins will complex with and activate the serine-threonine kinase p34^{cdc2} (refs 16–18) during the G₂/M phase transition. The p34^{cdc2}–cyclin complex is a constituent of the maturation promoting factor^{16–19} and cyclin is necessary before it can initiate mitosis and meiosis^{8–11}. Activation of maturation promoting factor coincides with maximal histone H1 kinase activity of p34^{cdc2} (refs 9, 12, 16 and 17) and with the dephosphorylation of its tyrosine residues²⁰. Human cyclin B migrates with a molecular weight of 62,000 (62K) in SDS-PAGE^{12–17}, and p34^{cdc2} is found to associate with a 60K protein in a complex which has maximal histone H1 kinase activity in interphase²¹. In extracts of frog eggs, cyclin is the only protein whose *de novo* synthesis is required for mitosis¹¹, and in isolated *Xenopus* oocytes, injection of cyclin mRNA is sufficient for induction of meiosis⁸. The degradation of cyclins is necessary for the cell to exit from mitosis¹¹. Three Lys-Tyr, three Lys-Lys and one Arg-Arg sequences are present in the human cyclin A protein, and might serve as cleavage sites for trypsin-like proteolytic enzymes¹⁰. The amount of cyclin in the cell has been shown to be regulated post-translationally in invertebrates^{6,7}, but the expression of human cyclin B is regulated both during and after transcription¹².

The interplay of cyclins with genes involved in tumorigenesis is illustrated in the phosphorylation, by maturation promoting factor of p60^{c-src} specifically at those residues phosphorylated during mitosis^{33,34}. The retinoblastoma gene product, a potential inhibitor of cell proliferation, also has its phosphorylation pattern modulated during the cell cycle^{36,38}, and phosphorylation of the large T antigen of SV40 by p34^{cdc2} stimulates the replication of virus³⁵.

The identification of a human cyclin A gene as a target for HBV integration in a hepatocellular carcinoma has to be considered in the light of hepadnavirus-related liver carcinogenesis.

Indeed, the importance of an HBV chronic infection in the development of human hepatocellular carcinoma has been conclusively established by epidemiological studies^{22–24} but evidence supporting a direct role for the virus in cell transformation is still scarce. The implications of viral DNA integration are controversial. In woodchuck hepatocellular carcinoma, the woodchuck hepatitis virus often acts by *myc* deregulation following viral insertion²⁵. In humans, HBV integration frequently induces deletions and translocations in a seemingly non-specific way^{3,26–28}. It has been suggested that integration on chromosome 11 (ref. 3) as well as rearrangement of a locus on chromosome 4q (refs 29, 30) could occur at a nonrandom rate. Integration of HBV in the vicinity of a cellular gene has so far only been demonstrated in one tumour, where the viral DNA interrupted the coding region of the retinoic acid receptor β (refs 31, 32). Our present observation shows that analysis of HBV integration sites can, in fact, identify cellular genes essential for cell growth, but it may be appropriate to analyse tumours which, like the one we have studied here are (1) in an early stage of cancer growth, so that chromosomal rearrangements are minimized, (2) associated with none of the histological features of chronic hepatitis and cirrhosis in the non-tumorous liver, where HBV might have acted only by inducing cirrhosis, a potentially pre-malignant condition, and (3) which show evidence for clonal proliferation of a cell containing only one HBV integration, which is absent in adjacent liver tissue.

A viral insertion interfering with the function of the cyclin A gene would be expected to severely affect the regulation of cell growth. Our preliminary results indicate that there is an increased level of cyclin A mRNA in the tumour compared with non-tumour tissue. Studies are in progress to determine how HBV integration has modified cyclin A expression in the tumour and to provide further insight into the role of cyclin A in normal and malignant cell growth. □

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Paternal origin of new mutations in Von Recklinghausen neurofibromatosis

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VON Recklinghausen neurofibromatosis (NF-1)¹⁻⁴ is a common autosomal dominant disorder. The estimated new mutation rate (1×10^{-4}) is one of the highest for a human disorder¹. Here we report that in 12 of 14 families we have analysed, the new mutation is of paternal origin. This result is similar to that recently obtained for retinoblastoma^{5,6}. In other genetic disorders that show a bias towards paternal origin of new mutations, there is a marked increase in the incidence of mutations with paternal age⁷, consistent with the mutations arising from replication errors in mitosis of spermatogonial stem cells. In retinoblastoma and NF-1, however, such paternal age effects are slight or absent^{3,7,8}. The mechanism or timing of germline mutation could therefore be different in the two cases.

Fourteen three-generation families were identified in which a new mutation to NF-1 had occurred in a single individual in the second generation. DNA typing of these families for loci closely linked to NF-1 (results summarized in Table 1 and Fig. 1) showed that in 12 of the 14 cases, it was highly probable that the new mutation had occurred on the paternal chromosome. The combined results give a maximum likelihood estimate of 0.84 for the fraction of mutations that are of paternal origin. Using a χ^2 approximation to the generalized likelihood ratio test, the null hypothesis of no sex difference in mutation rate at the NF-1 gene can be rejected with confidence ($\chi^2 = 7.13$, 1

TABLE 1 Probability of paternal or maternal origin of NF-1 mutation

Family and source (*)	P (paternal)	P (maternal)
ICR 25	0.9925	0.0075
ICR 26	0.0300	0.9700
ICR 28	0.0400	0.9600
ICR 29	0.9800	0.0200
ICR 30	0.9796	0.0204
ICR 31	0.9800	0.0200
UTK 1611	0.9999	0.0001
UTK 1614	0.9999	0.0001
UTK 1624	0.9999	0.0001
CAR 213	0.9100	0.0900
CAR 214	0.9971	0.0029
CAR 216	0.9100	0.0900
CAR 217	0.9800	0.0200
CAR 218	0.9700	0.0300

Probability calculated from multilocus genetic data. Family members were examined by an experienced clinician using standard criteria for the diagnosis of NF-1^{1,3} to confirm that both parents of the presumed new mutation had no stigmata of NF-1, and that one or more unequivocally affected children in the third generation could be used to determine the chromosome that carried the NF-1 mutation. (In one family, CAR 214, the third generation contained only two unaffected children aged 7 and 14 years. Because of the very high penetrance of the NF-1 gene, they could nevertheless be used to infer which chromosome carried the NF-1 mutation.) DNA was extracted from blood, digested with restriction enzymes and analysed by Southern blotting with one or more of the following probes closely linked to the NF-1 locus: EW301, p17H8, HHH202, pTH17.19, β -crystallin (β 8.2), p11-2C11.5, p11.2C11.7, EW206, EW207, EW204, VAW210, VAW211, VAW212, VAW215, CRI-L581 and CRI-L946 (for details, see refs 4, 10, 14). Paternity was confirmed in the ICR and CAR families using locus-specific minisatellite probes¹⁵⁻¹⁸; in the UTK families, over 80 polymorphic probes have been tested without inconsistency. The chromosome on which the new mutation originated was inferred from the haplotypes in each family (for example, see Fig. 1), and the probability of an erroneous result due to recombination calculated in each case^{10,14}.

* ICR, Institute of Cancer Research, London; UTK, University of Utah; CAR, Welsh National School of Medicine, Cardiff.

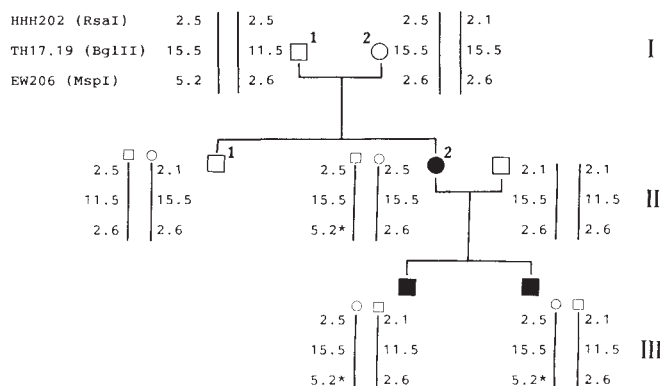


FIG. 1 Determination of the parental origin of a new NF-1 mutation. Family ICR 25. Genotypes are shown for three loci, two recognized by the probes pHH202 and pTH17.19 which are proximal to NF-1, and one by probe pEW206, which is distal. Alleles are represented as the sizes in kilobases of the corresponding DNA fragments. The results with probe pEW206 indicate that the chromosome that carried the NF-1 mutation (marked by an asterisk in generations II and III) was inherited from the grandfather I-1. Haplotypes indicate that for I-1 and II-2, no recombination occurred in the region between the flanking markers TH17.19 and EW206 in association with the mutation to NF-1. ■ and ●, NF-1.

degree of freedom; $P < 0.01$). The average age at birth of the affected child of the fathers from whom the new mutation originated was 29.8 years (range, 24–41 years), which is not significantly different from the average paternal age of 30.02 years for live births in the population of England and Wales for the period 1961–65. To determine whether new mutations were associated with genetic recombination, the haplotypes of five unaffected siblings of new mutations in three families were scored (all paternal in origin). There was no evidence of crossing-over in the haplotyped region for any sibship.

A bias towards paternal origin of new mutations has been inferred for the X-linked disorders haemophilia A and Lesch-Nyhan syndrome, and for some autosomal disorders, for example achondroplasia. Each of these shows a strong paternal age effect—that is, the incidence of new mutations increases with paternal age⁷. This is most easily explained if most new mutations occur at mitosis in a spermatogonial stem cell which undergoes asymmetric division, one daughter persisting as the stem cell. In this way, the stem cell population can accumulate mutations with time. The paternal origin of the mutations is explained because only spermatogonial stem cells, and not oogonia, undergo mitotic divisions after birth⁷. But in NF-1, the paternal age effect is slight or absent^{1,7,8}.

NF-1 resembles Duchenne muscular dystrophy in its high mutation rate. The high mutation rate in Duchenne muscular dystrophy could simply reflect the large size of the gene, but misalignment of repeated elements of sequence at meiosis with subsequent deletion and duplication has also been suggested as an explanation⁹. A meiotic origin of most NF-1 mutations seems unlikely as the recombination frequency in the region of the NF-1 locus is considerably greater in females than in males¹⁰, which in the absence of other modifying factors would be

expected to result in a maternal bias in the origin of mutations. In the three families that we could assess, there was no evidence of recombination, whether meiotic or mitotic, in association with the new NF-1 mutation. This is consistent with a recent report¹¹ that unequal exchange between homologous chromosomes at meiosis does not explain the high mutation rate at the minisatellite locus λ MS1.

NF-1 resembles retinoblastoma in that both show a predominance of paternal new mutations, but little or no paternal age effect^{7,8}. Equal transmission of the mutant gene through the male or female germ line in established families in each case, excludes selection against mutations carried on maternally derived chromosomes as an explanation for the paternal origin of new mutations. We conclude: (1) that most NF-1 mutations probably arise either at mitosis in a cell that is not a self-renewing stem cell, or independently of mitosis, for example, in mature sperm; and (2) that paternal chromosomes are more susceptible to this mutation than are maternal chromosomes. The reason for this is unclear, but could be related to the differences in methylation of paternal and maternal germ lines believed to be associated with genomic imprinting¹². The new high mutation rate at the NF-1 locus could reflect either the large size of the

gene, or the presence within it of sequences that are highly susceptible to mutation. □

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Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia

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MALIGNANT hyperthermia (MH) is a potentially lethal condition in which sustained muscle contracture, with attendant hypercatabolic reactions and elevation in body temperature, are triggered by commonly used inhalational anaesthetics and skeletal muscle relaxants¹. In humans, the trait is usually inherited in an autosomal dominant fashion², but in halothane-sensitive pigs³ with a similar phenotype, inheritance of the disease is autosomal recessive or co-dominant. A simple and accurate non-invasive test for the gene is not available and predisposition to the disease is currently determined through a halothane- and/or caffeine-induced contracture test on a skeletal muscle biopsy^{4,5}. Because Ca^{2+} is the chief regulator of muscle contraction and metabolism, the primary defect in MH is believed to lie in Ca^{2+} regulation^{1,6}. Indeed, several studies indicate a defect in the Ca^{2+} release channel of the sarcoplasmic reticulum^{7–12}, making it a prime candidate for the altered gene product in predisposed individuals. We have recently cloned complementary DNA and genomic DNA encoding the human ryanodine receptor¹³ (the Ca^{2+} -release channel of the sarcoplasmic reticulum) and mapped the ryanodine receptor gene (*RYR*) to region q13.1 of human chromosome 19 (ref. 14), in close proximity to genetic markers that have been shown to map near the MH susceptibility locus in humans¹⁵ and the halothane-sensi-

tive gene in pigs¹⁶. As a more definitive test of whether the *RYR* gene is a candidate gene for the human MH phenotype, we have carried out a linkage study with MH families to determine whether the MH phenotype segregates with chromosome 19q markers, including markers in the *RYR* gene. Co-segregation of MH with *RYR* markers, resulting in a lod score of 4.20 at a linkage distance of zero centimorgans, indicates that MH is likely to be caused by mutations in the *RYR* gene.

The MH families included in the study were those in which two or more offspring were at risk of inheriting the MH gene from an affected parent. The chromosome 19 restriction fragment length polymorphisms (RFLPs) used in the linkage study are shown in Table 1. These included several known chromosome 19q polymorphic markers and 11 new RFLPs at the human *RYR* locus that were discovered using cloned *RYR* cDNA probes.

To test for co-segregation with the MH phenotype, it was crucial to have an accurate assessment of MH status in the individual members of the families under study. This assessment was carried out on biopsied muscle strips exposed to caffeine, halothane, or caffeine plus halothane, and is based on the differential contractile response of normal and MH muscle⁵. Families who did not fit the standard criteria for autosomal dominant inheritance were not used for the linkage study. Of 21 families examined, nine were informative for one or more *RYR* polymorphisms. Eight others that were not informative for *RYR* markers were informative for a set of flanking markers on chromosome 19, and therefore provided useful information. The *RYR* segregation data for two typical families is shown in Fig. 1a, b.

In family 5 (Fig. 1a), there are three segregating *RYR* polymorphisms in three children of an affected mother. The mother is heterozygous for the three markers and the father is homozygous for these markers, making it an ideal family for study. According to the biopsy, the three children are all classified as carriers of the MH susceptibility gene and, according to the RFLP markers, all three have received the same copy of the *RYR* gene from their mother. In family 12 (Fig. 1b), there are four children of an affected father. The father is heterozygous for one *RYR* marker and for the *ApoCII* marker on chromosome 19. Biopsy results indicate that two children received the defective MH gene and two others received the normal allele at the MH locus. The two normal girls received the 4.4 kilobase (kb) *HRR3* allele and the 3.8 kb *ApoCII* allele from their affected father, whereas the two MH-positive children received the opposite allele at each of the two loci.

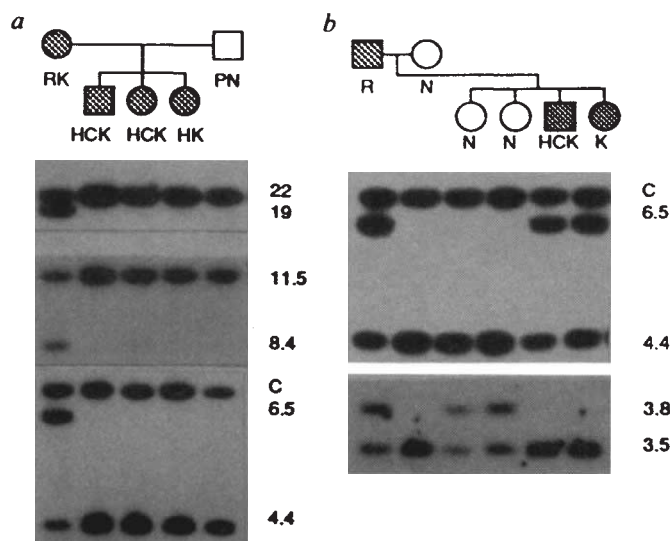


FIG. 1 Segregation of *RYR* gene markers and flanking markers in families 5 and 12. Diagnosis of MH status was carried out using the caffeine-halothane contracture test described previously⁵.

METHODS. Surgical biopsies of *Vastus lateralis*, *rectus abdominis* or *gracilis* muscles were removed under general anaesthetic (nitrous oxide, Innovar and midazolam). Biopsies, trimmed free of fat, were divided into 100–300 mg (1–5 mm by 10–20 mm) strips and secured at one end by a silk suture to an electrode frame in Krebs–Ringer bicarbonate solution, pH 7.4, at 37 °C, and aerated with 95% O₂ and 5% CO₂. Strips were secured at the other end to a force displacement transducer to measure muscle tension. Twitch heights and baselines were established during a 20 min pre-test interval. Individuals were classified according to their responses to the halothane test (H), the caffeine test (C) and the caffeine plus halothane test (K). A positive halothane test was one that gave a contracture >0.3 g in 1% halothane or >0.7 g in 3% halothane. For the caffeine test, 0.5 mM caffeine was added and, at 4-min intervals, the dose was doubled to a final concentration of 32 mM. The dose of caffeine required to raise the resting tension (baseline to plateau) by 1 g was noted. Values above 4.0 were considered normal, values below 4.0 as abnormal. In the caffeine-plus-halothane test, muscle strips were equilibrated for 15 min with 1% halothane. Caffeine was added at 0.25 mM and doubled every 4 min to a dose of 32 mM. Caffeine concentrations required to raise the resting tension by 1 g were considered as normal when >0.5 mM and abnormal if <0.35 mM. Values in between were considered as ambiguous. Family members are labelled with an R if they have had an MH reaction and with H, C, or K if they tested positive for the halothane test, the caffeine test or the halothane + caffeine test, respectively. Individuals labelled N tested normal in the contracture tests; PN is presumed normal. Southern blot autoradiographs for selected informative markers are shown with the DNA tracks aligned below the corresponding family member. In family 5 (a), the three informative markers were HRR3 (*Hind*III alleles of 19 and 22 kb); HRR4 (*Bcl*I alleles of 11.5 kb and 8.4 + 2.9 kb; the 2.9 kb band is not shown) and HRR3 (*Pvu*II alleles of 6.5 kb and 4.4 + 1.9 kb; the 1.9 kb band is not shown). In family 12 (b), two informative markers are HRR3 (*Pvu*II alleles of 6.5 kb and 4.4 + 1.9 kb; the 1.9 kb band is not shown) and ApoCII (*Taq*I alleles of 3.8 and 3.5 kb).

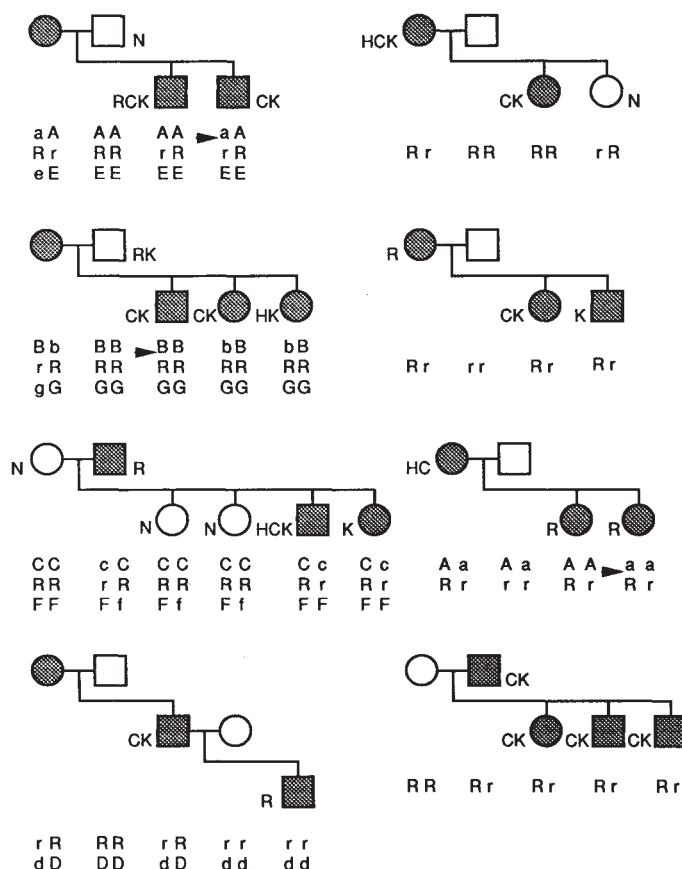


FIG. 2 Segregation data from eight of the nine families informative for *RYR* gene probes. Only family members who were tested for predisposition to MH by muscle biopsy are presented. Individuals are labelled R, N, H, C and K as in Fig. 1. Family numbers are: left, top to bottom; 1, 5, 12, 20 and right, top to bottom; 23, 24, 27, 30. In each family, one ryanodine receptor gene marker is shown with flanking markers, where informative. Flanking markers are designated as A, a, B, b, ..., G, g corresponding to the major (most frequent) and minor allele of the markers A, B, ..., G described in Table 1. The *RYR* alleles are designated simply R and r, irrespective of which probe and enzyme combinations proved to be informative. When multiple *RYR* markers were informative, they all behaved identically in the family, with no recombination taking place between them. In children of an affected parent, the chromosomes are arranged with the maternal chromosome to the left and the paternal to the right. The affected grandmother in family 20 and the affected father in family 30 were each part of larger pedigrees in which the MH gene was segregating. No recombinants between the MH gene and the *RYR* markers were found. Three recombinants for flanking markers were revealed, one at marker A in family 1 (one marked with arrow, although as phase in the mother is not established, either child could be the recombinant), one at marker B in family 5 (arrow) and one at marker A in family 27 (arrow, although as phase in mother is not established, either child could be recombinant). A ninth family, whose data were added to the lod score table after submission of this work, had three children (N; CK; R) of an affected mother (K). The two affected children received the R allele and a normal child received the r allele from the heterozygous mother. The mother had three MH-positive siblings who were not available for linkage study.

A summary of the segregation data for eight of the nine *RYR* informative families is shown in Fig. 2. Data are presented for one *RYR* marker and for flanking markers when possible. In most families, more than one *RYR* marker was informative, but as a recombinant between *RYR* markers was never observed, multiple *RYR* polymorphisms were treated as a single marker for linkage analysis. In each family, the affected parent was heterozygous for the *RYR* polymorphism and the unaffected parent was homozygous for this marker, making all of the children fully informative. In each of the nine families, there was complete co-segregation of one *RYR* allele with the MH phenotype. Flanking markers also displayed tight linkage. We

observed only three recombinants with these markers, two between MH and marker A (probe 17.4) in families 1 and 27 and another between MH and marker B (probe HW60) in family 5. In these families, as in the others, the *RYR* probe was non-recombinant.

Table 2 presents a summary of the linkage information in the form of a lod score table for all of the markers used in the study. The MH gene segregated with chromosome 19 markers and a clear linkage was found between the MH and the *RYR* genes. The lack of recombination between the MH gene and the *RYR* gene (Fig. 2) generates a peak lod score of 3.59 at a linkage distance of zero centimorgans (cM; 1 centimorgan is the genetic

TABLE 1 Ryanodine receptor probes and flanking probes on chromosome 19

Marker (Fig. 2)	Locus*	Probe name†	RFLP enzyme	Polymorphic band sizes	Allele frequency	Ref.
A	<i>D19S29</i>	17.4	<i>MspI</i>	3.5/5.5 · 3.6(C)‡		17
B	<i>D19S13</i>	HW60	<i>BglII</i>	6.7/15.0		18
			<i>TaqI</i>	2.6/5.5		
C	<i>D19S28</i>	5B18	<i>TaqI</i>	1.7/2.7		19
R	<i>RYR</i>	HRR1-1600	<i>BanI</i>	6.0/13.0	0.22/0.78	
		HRR3-1000	<i>HindIII</i>	19.0/22.0	0.04/0.96	
			<i>PvuII</i> §	6.5/4.4, 1.9	0.25/0.75	
			<i>BamHI</i> §	19.0/14.0, 5.0	0.75/0.25	
		HRR3-1200	<i>PvuII</i>	3.9, 1.9/5.8	0.11/0.89	
		HRR4-2400	<i>TaqI</i>	1.8/1.1, 0.7 3.5(C)	0.20/0.80	
			<i>BclI</i>	11.5/8.4, 2.9	0.78/0.22	
				4.6, 3.0(C)		
		HRR5-3800	<i>EcoRV</i>	28.0/2.5 11.0(C)		
			<i>EcoRI</i>	24.0/16.0, 9.0	0.14/0.86	
			<i>TaqI</i>	1.9/1.6, 0.3		
			<i>MspI</i>	1.8/2.2		
D	<i>D19S18</i>	PM6.7	<i>MspI</i>	1.7/3.0		20
			<i>EcoRI</i>	25.0/40.0		
E	<i>D19S15</i>	JSB6	<i>TaqI</i>	4.5/2.7		21
F	<i>D19S16</i>	JSB11	<i>TaqI</i>	6.5/8.5		22
			<i>MspI</i>	1.7/1.6		
G	<i>APOCII</i>	APOCII	<i>TaqI</i>	3.5/3.8		23

* The order of the flanking probes is as determined by Schonk *et al.*²⁴ and the position of the *HRR* gene relative to these markers was determined by MacKenzie *et al.*¹⁴.

† HRR1 to HRR5 are probes from the human ryanodine receptor cDNA described by Zorzato *et al.*¹³. Their localization on the cDNA map is as follows: HRR1-1600, bases 13,602 to 15,243; HRR3-1000, bases 8,515 to 9,554; HRR3-1200, bases 9,549 to 10,851; HRR4-2400, bases 6,125 to 8,493; HRR5-3800, bases 2,381 to 6,130.

‡ Constant hybridizing band.

§ In total linkage disequilibrium with each other and were treated as a single marker.

TABLE 2 Linkage with the MH locus*

Locus	Probe	$\theta_{\max}$	$z_{\max}$	θ							$N^{\dagger}$	$F^{\ddagger}$
				0.00	0.01	0.05	0.1	0.2	0.3	0.4		
<i>D19S29</i>	17.4	0.33	0.025	-104.19	-3.80	-1.53	-0.68	-0.10	0.02	0.02	21	9
<i>D19S13</i>	HW60	0.10	1.52	-98.84	0.44	1.37	1.52	1.25	0.75	0.26	24	9
<i>D19S28</i>	5B18	0.08	1.45	-97.88	0.95	1.42	1.43	1.11	0.67	0.24	15	5
<i>RYR</i>	HRR	0.00	4.20	4.20	4.10	3.71	3.21	2.20	1.20	0.39	23	9
<i>D19S18</i>	PM6.7	0.15	0.65	-198.00	-0.88	0.28	0.59	0.60	0.39	0.15	19	8
<i>D19S16</i>	JSB11	0.08	1.15	-98.19	0.65	1.12	1.13	0.86	0.48	0.14	14	5
<i>ApoCII</i>	ApoCII	0.10	0.99	-98.19	0.36	0.90	0.99	0.84	0.53	0.20	11	4

* Results are expressed as a lod scores (Z) at recombination fraction values (θ).

† Number of meioses.

‡ Number of informative families.

distance corresponding to a recombination frequency of 1%) and is entirely consistent with identity of the two genes. Our genetic linkage data, combined with the physiological data, which are fully consistent with a Ca^{2+} release channel defect in individuals predisposed to malignant hyperthermia, make a powerful argument that the basic defect in MH results from mutations in the *RYR* gene. The fact that the porcine halothane-sensitive gene segregates with the *GPI* locus¹⁶, and that this locus maps in humans to 19q very close to the *RYR* gene¹⁴, lends further support to the view that mutations in the ryanodine receptor gene are also responsible for the halothane-sensitive phenotype in pigs. Definitive proof that *RYR* is the gene responsible for MH in man and pigs will await the description of *RYR* mutations in MH-susceptible individuals. □

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Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2

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MALIGNANT hyperthermia (MH) is an inherited human skeletal muscle disorder and is one of the main causes of death due to anaesthesia¹. The reported incidence of MH varies from 1 in 12,000 in children to 1 in 40,000 in adults^{2,3}. MH is triggered in susceptible people by all commonly used inhalational anaesthetics; it is characterized by a profoundly accelerated muscle metabolism, contractures, hyperthermia and tachycardia^{1,4,5}. Susceptibility to MH (MHS) is predicted by contracture tests on muscle tissue obtained by biopsy^{6,7}. An almost identical disorder known as porcine MH exists in pigs^{8,9}. The genetics of the porcine syndrome have been extensively studied¹⁰; the locus controlling expression of porcine MH is genetically linked to the glucose phosphate isomerase locus (*GPI*)¹¹. In man, *GPI* has been mapped to the q12-13.2 region of chromosome 19 (refs 10-12). We have now investigated genetic linkage in several extended Irish pedigrees in which MHS is segregating as an autosomal dominant trait. Here we show linkage between MHS and DNA markers from the *GPI*

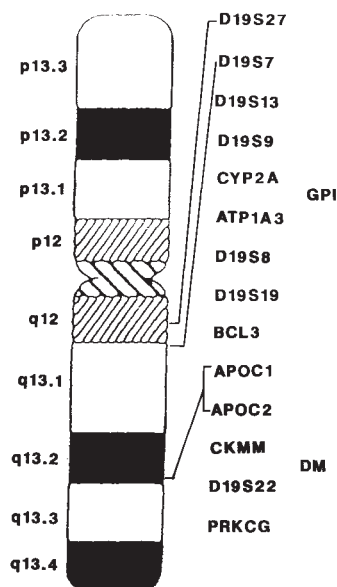


FIG. 1 Linkage map of 14 markers on chromosome 19q. Data are from a multipoint analysis of the CEPH pedigrees¹⁹ with the location of *GPI* from somatic cell hybrid data¹⁸ and the position of the *DM* locus distal to *CKMM* from analysis of recombinant family members²⁶⁻²⁸.

region of human chromosome 19 with a maximum log likelihood ratio (lod score) of 5.65 at the *CYP2A* locus. These results indicate that human and porcine MH are most probably due to mutations in homologous genes, and also provide a potentially accurate and noninvasive method of diagnosis for MHS.

A standardized test (based on contractures induced in muscle *in vitro* by caffeine and halothane) for the investigation of MH susceptibility in individuals has been established and validated by the European MH group^{7,15}. This test allows the following diagnoses to be made: MH-susceptible (MHS), MH-negative (MHN), and MH-equivocal (MHE).

The locus controlling expression of porcine MH is very tightly linked to the *GPI*¹¹, alpha 1-B glycoprotein (*GA1B*)¹⁶ and H blood group antigen loci¹⁷. This linkage group, spanning about 12 centimorgans (cM; a centimorgan is equivalent to 1% recombination between two loci and corresponds to $\sim 1 \times 10^6$ base pairs of DNA), is highly conserved in humans and maps to chromosome 19q12-13.2 (ref. 16). This region of chromosome 19 contains the myotonic dystrophy (*DM*) locus and several well-defined polymorphic loci including an apolipoprotein gene cluster (*APOC1*, *APOC2* and *APOE*), *BCL3*, *CYP2A* and

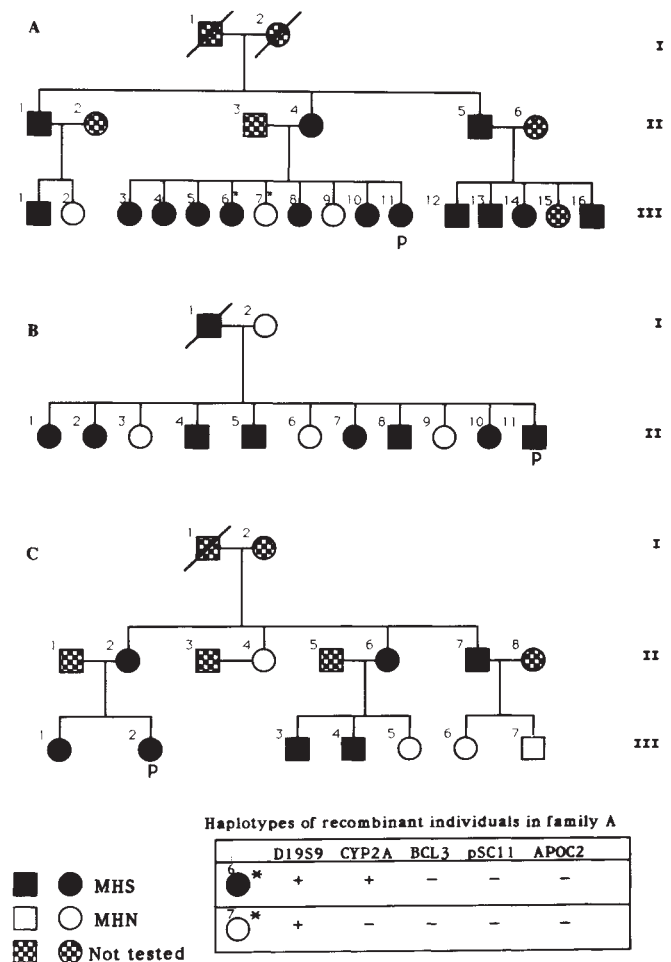


FIG. 2 Pedigrees of three Irish MHS families. Individuals were diagnosed as MHS or MHN by the standardized European contracture test⁷. There were no MHE individuals in these families. In each pedigree the proband (P) suffered a clinical episode of MH during anaesthesia and recovered. DNA was isolated from peripheral blood of all individuals for genotyping with polymorphic DNA markers. The alleles of the markers *D19S9*, *CYP2A*, *BCL3*, *pSC11* and *APOC2* were combined into haplotypes for all members of family A. The haplotypes of the two recombinant individuals III-6 and III-7 in family A are shown at the bottom and are denoted by an asterisk in the pedigree. +, Affected individual's genotype; -, unaffected individual's genotype.

TABLE 1 Lod score for MHS versus *D19S9*, *CYP2A* and *APOC2** markers

Equal male and female recombination fractions										
	0.00	0.001	0.01	0.05	Recombination frequency (θ)					
					0.10	0.15	0.20	0.25	0.30	0.40
<i>D19S9</i> vs MH	2.07	2.10	2.30	2.50	2.42	2.23	1.98	1.68	1.35	0.59
<i>CYP2A</i> vs MH	0.74	0.74	0.72	0.64	0.55	0.45	0.36	0.27	0.18	0.05
<i>APO*</i> vs MH	2.60	2.68	3.07	3.59	3.56	3.31	2.95	2.45	2.00	0.84
Female θ varying only (male $\theta=0.5$)										
	0.00	0.001	0.01	0.05	Recombination frequency					
					0.10	0.15	0.20	0.25	0.30	0.40
<i>D19S9</i> vs MH	0.21	0.25	0.47	0.80	0.88	0.85	0.77	0.65	0.51	0.19
<i>CYP2A</i> vs MH	0.08	0.08	0.08	0.07	0.05	0.04	0.03	0.02	0.01	0.00
<i>APO*</i> vs MH	0.70	0.73	0.95	1.26	1.31	1.25	1.15	1.00	0.84	0.45
Male θ varying only (female $\theta=0.5$)										
	0.00	0.001	0.01	0.05	Recombination frequency					
					0.10	0.15	0.20	0.25	0.30	0.40
<i>D19S9</i> vs MH	1.54	1.54	1.51	1.39	1.24	1.08	0.91	0.73	0.55	0.19
<i>CYP2A</i> vs MH	0.37	0.37	0.36	0.32	0.26	0.21	0.16	0.12	0.08	0.02
<i>APO*</i> vs MH	0.17	0.22	0.68	1.35	1.53	1.50	1.36	1.18	0.92	0.37

Lod scores were calculated with the LINKAGE program²¹ for combined sexes ($\Theta_{\text{male}} = \Theta_{\text{female}}$), male recombination alone ($\Theta_{\text{male}}, \Theta_{\text{female}} = 0.5$) and female recombination alone ($\Theta_{\text{male}} = 0.5, \Theta_{\text{female}}$). The frequency of the *MHS* mutant allele was kept at 0.001; lod scores calculated with *MHS* frequencies of 0.0001 or 0.01 did not appreciably vary. Two 'liability' classes were defined for *MHS*: class 1 (patients who had undergone an MH crisis) defined with complete penetrance; and class 2 (*MHS*, unaffected and unknown individuals) with reduced penetrance (0.99) and a 1% phenocopy rate. The latter liability class allows for potential inaccuracies in diagnosis with the muscle biopsy test. Three probes (*APOC2*, *pSC11* and *BCL3*) have been physically mapped to a 300-kilobase interval²⁵ and lod scores for these three markers were combined into a single haplotype (*APO**).

*D19S9*¹⁸. The order of 14 markers spanning 63 cM on 19q is known (Fig. 1) from both physical and genetic mapping studies prompted by research to isolate the *DM* gene^{18,19}. Because of the phenotypic similarity of human and porcine MH, it seemed likely that the human MH locus would map to 19q. We tested this possibility by investigating linkage between *MHS* and markers that span 19q12–13.2 in three large Irish families in which *MHS* is segregating as an autosomal dominant trait (Fig. 2). We typed the markers *D19S9*, *BCL3*, *pSC11* and *APOC2* in these families and calculated the two-point lod scores (Table 1).

The maximum two-point lod score generated for these markers was 3.67 at recombination fraction $\theta = 0.073$ for the haplotype of markers *APOC2*, *pSC11* and *BCL3* (Table 1). The genetic distance between *D19S9* and *APOC2* has been estimated to be 20 cM from linkage analysis in the CEPH panel of families^{19,20}

and no sex difference in recombination is seen across this interval²⁰. Several polymorphic loci map into this interval (Fig. 1), and one of these, *CYP2A*, was informative in family A (Fig. 2). Multipoint joint likelihood calculations were computed with the LINKAGE computer program²¹, and are shown as a location map (Fig. 3). The maximum lod score in the multipoint analysis using *CYP2A* was 5.65 at $\theta = 0$. The multipoint analysis indicates that *D19S9* and *APOC2* flank the *MHS* locus.

Inspection of the haplotypes in family A shows that markers proximal to *BCL3* segregate with *MHS* in affected individual III-6. By contrast, the allele of the *D19S9* marker that usually segregates with *MHS*, segregates with *MHN* in individual III-7 (Fig. 2). Taken together, these two recombination events confirm the mapping of *MHS* and *CYP2A* into the interval between *D19S9* and *APOC2*. In somatic cell hybrids, *CYP2A* and *GPI* cosegregate¹⁸, which, with the data presented here, strongly indicates that human MH and porcine MH are homologous disorders.

Although the defect responsible for *MHS* is not known, biochemical evidence indicates that the skeletal muscle ryanodine receptor is abnormal in pigs affected with MH²². The human ryanodine receptor gene has recently been localized to the cen-q13.2 region of chromosome 19 (ref. 23), and in the accompanying letter MacLennan *et al.*²⁴ show that polymorphic markers for this gene cosegregate with the human MH susceptibility trait. These data, taken together with the linkage studies presented here, indicate that the ryanodine receptor gene is a highly likely candidate for the *MHS* gene. □

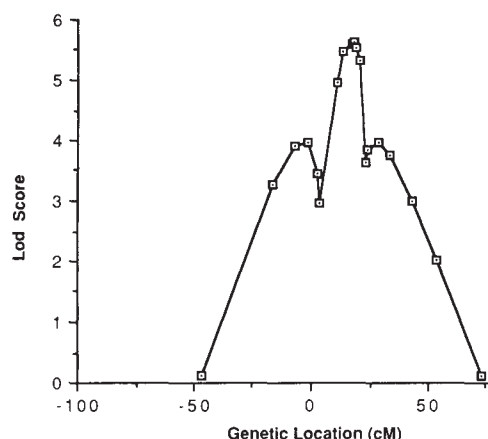


FIG. 3 Location map summarizing location scores ($\log_{10}$ likelihood difference) calculated for *MHS* at various map positions in a fixed marker map. Joint likelihoods were calculated for the six loci simultaneously. Here the support for linkage of *MHS* is calculated at various map locations in a fixed map of polymorphic markers. The most likely location for *MHS* is at *CYP2A* with a lod score of 5.65. On the x-axis *APOC2* is arbitrarily set at 0 cM, *D19S9* is at +20 cM, and *CYP2A* at +15 cM, based on linkage maps published for this region^{19,20}.

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Vasoactive intestinal peptide regulates mitosis, differentiation and survival of cultured sympathetic neuroblasts

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ALTHOUGH acute, millisecond-to-millisecond actions of neurotransmitters are well documented, diverse longer-term effects have been discovered only recently^{1–5}. Emerging evidence indicates that these signals regulate a variety of neuronal processes, from phenotypic expression to neurite outgrowth^{6–19}. Here we show that a single putative transmitter, vasoactive intestinal peptide, can exert multiple, long-term effects simultaneously: it stimulates mitosis, promotes neurite outgrowth and enhances survival of sympathetic neuron precursors in culture. As the peptide seems to be a normal presynaptic transmitter in the sympathetic system^{19–25}, synaptic transmission may exert hitherto unexpected effects.

To study early neuronal ontogeny, we developed a fully defined, dissociated cell culture system composed of an enriched population of sympathetic precursors or neuroblasts²⁶. Embryonic neuroblasts, derived from superior cervical ganglia from rats of 15.5 days' gestation, were identified immunocytochemically by detection of neurofilament and the catecholamine biosynthetic enzyme, tyrosine hydroxylase²⁶. Earlier work indicated that these cells enter the mitotic cycle in culture^{26,27}. We investigated the effect of vasoactive intestinal peptide (VIP) using this system, because in the adult rat the peptide is localized to presynaptic fibres that innervate these ganglion neurons^{19,22–24}. VIP elicits a striking twofold increase in [³H]thymidine incorporation per culture, indicating that the peptide increases neuroblast DNA synthesis (Fig. 1a). This increase in incorporation is comparable to that stimulated by the insulin growth factors in this system²⁶ (Fig. 1b). Moreover, increased synthesis reflects a larger number of blasts entering mitosis, not simply an enhanced incorporation by a pre-existent mitotic population: VIP more than doubles the percentage of tyrosine hydroxylase-positive cells incorporating thymidine into nuclei (Fig. 1c). Consequently, VIP increases the mitotic population *in vitro*. Finally, the effects of VIP on mitosis are highly specific as secretin and glucagon, two closely related peptides, do not alter DNA synthesis (data not shown).

In addition to regulating mitosis, VIP markedly increases the number and complexity of neurites. The peptide evokes a six-

TABLE 1 VIP content of embryonic day-15.5 ganglion

Tissue	VIP (pg per µg protein)
SCG	1.12 ± 0.059 (3.07 ± 0.093 pg per ganglion)
Liver	0
Adult ileum	0.62 ± 0.117

Ganglia were dissected, pooled (10–20 per sample) and frozen at –70 °C. Adult ileum⁴⁹ and embryonic liver were examined as positive and negative assay controls, respectively. Tissues were extracted in 0.01 M HCl and radioimmunoassayed for VIP using a commercial kit (Peninsula). The lowest quantity of VIP detectable by this method was 1 pg. The antibody exhibited <0.001% cross-reactivity with other neuropeptides including VIP 1–12, peptide histidine-isoleucine or substance P at 10,000-fold molar excess. Values are corrected for recovery (80%), expressed as pg per µg acid-extractable protein and represent the mean ± s.e.m. of at least 4 samples.

fold rise in the number of process-bearing cells 10 h after plating (Figs 2a, b and 3a). The elevation is not due to selective survival of a process-bearing subpopulation, because VIP does not alter the overall cell number at this time. Further, the neurotogenic effects are sustained, because VIP also markedly increases the percentage of neuroblasts with long processes at 24 h (Figs 2c, d and 3b). Our observations suggest that VIP simultaneously promotes differentiation and mitosis in the same cultures.

During ontogeny, both precursor proliferation and cell survival contribute to the formation of stable neuronal populations^{28,29}. To investigate whether VIP influences neuroblast survival, we determined the cell numbers after a longer period in culture, namely 4 days. In fact, VIP exposure results in >300% increase in the number of cells per culture compared with the control (Fig. 3c). Virtually the entire surviving population expressed tyrosine hydroxylase, suggesting that VIP exerts effects on cells of the sympathetic lineage (data not shown). Moreover, even in the presence of the mitotic inhibitor aphidicolin (at 5 µg ml^{–1})³⁰, which inhibits [³H]thymidine incorporation by 80%, VIP treatment still increases cell numbers over the controls (control: 893 ± 174 cells per dish; VIP: 7,750 ± 638 cells per dish; differ at *P* < 0.001 by Student's *t*-test). Thus, in addition to influencing mitosis and differentiation, VIP seems to exert a trophic effect on sympathetic neuroblasts.

We characterized the specific nature of this effect on survival by testing other neuropeptides closely related to VIP. Secretin, peptide histidine-isoleucine and growth hormone-releasing factor, which are known to alter transmitter traits in the superior cervical ganglion (SCG)^{19,22,23}, have no trophic effects in culture; only VIP enhances neuronal survival (data not shown).

As VIP has a function in the mature sympathetic nervous system, we looked for VIP in embryonic day 15.5 SCG by radioimmunoassay of ganglia extracts (Table 1) and found significant amounts were present (2.3–3.0 pg per ganglion). This suggests that the transmitter is physiologically important during development.

The multiple effects of VIP on mitosis, process elaboration and cell survival could be mediated by direct action on neuroblasts or by indirect action through non-neuronal cells. In fact, non-neuronal cells made up less than 1.5% of the cells plated initially. Moreover, after four days of incubation, non-neuronal numbers did not increase in control medium or in the presence of VIP. Thus, the peptide did not promote division or survival of the non-neuronal population. The tiny number of non-neuronal cells and the rarity of non-neuron–neuron contacts indicates that VIP probably elicits neuroblast mitosis, differentiation and survival in culture directly.

The VIP concentrations necessary to exert these effects on sympathetic neuroblasts are higher than other reported *K_d* values for VIP binding in the nervous system, which range between 1 and 70 nM (refs 31, 32), but a low-affinity VIP receptor with an apparent *K_d* of 285 nM has been reported³³. This *K_d* value is comparable to the half-maximal VIP concentration

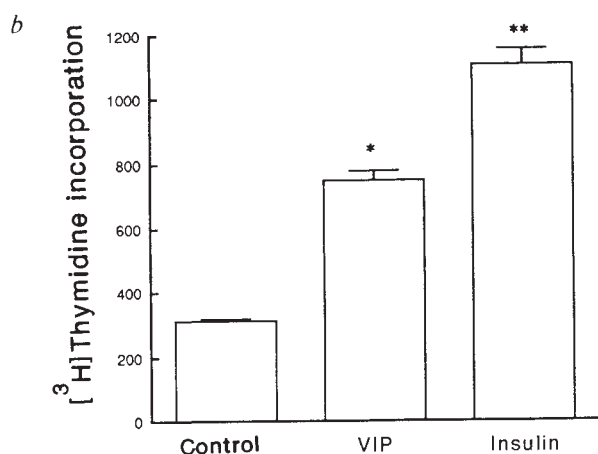
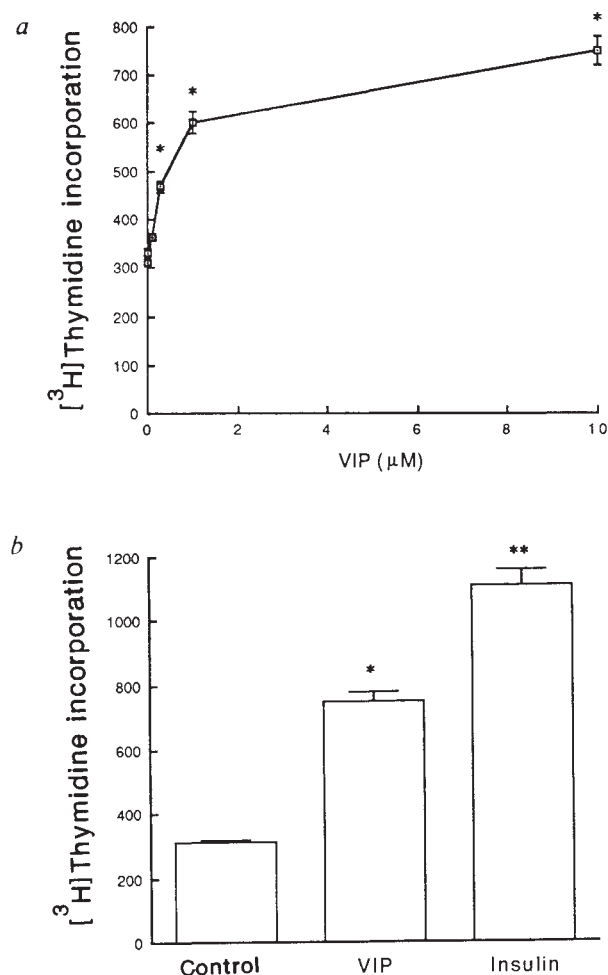
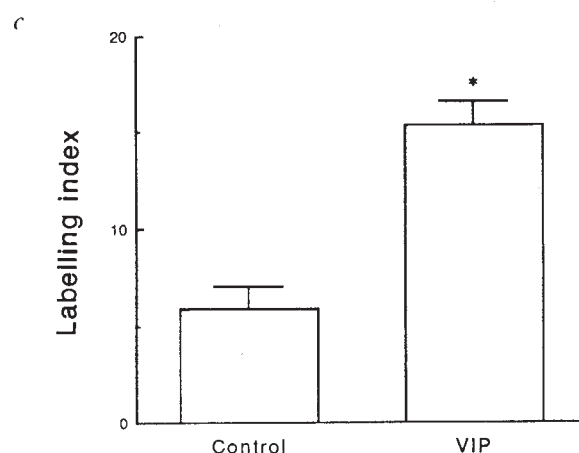


FIG. 1 a, Effect of VIP on [^3H]thymidine incorporation by cultured neuroblasts. Cultures were incubated for 48 h in control medium or in medium containing various concentrations of VIP. Each experimental value represents the mean incorporation of four culture wells and is expressed as mean c.p.m. $\pm$ s.e.m.



b, Comparison of effects of VIP (10 μM) and insulin (10 $\mu\text{g ml}^{-1}$) on [^3H]thymidine incorporation c, Effect of VIP on the labelling index of cultured neuroblasts. Cultures were incubated for 48 h without VIP (control) or with VIP (10 μM).

METHODS. Superior cervical ganglia from embryonic day-15.5 rats were dissociated and grown in fully defined medium on a poly-D-lysine and fibronectin substratum as previously described²⁶. Insulin, a previously defined mitogen in this system was omitted from the culture medium to avoid confounding effects²⁷. [^3H]thymidine (1 $\mu\text{Ci ml}^{-1}$) was added at 24 h of incubation. a, b, Incorporation was studied in 24-well plates using a semi-automatic cell collector. At 48 h, cells were incubated in Ca^{2+} - and Mg^{2+} -free saline G (ref. 26) containing trypsin (0.25%) and EDTA (0.5 mM) at 37 $^{\circ}\text{C}$ for 20 min and collected on glass fibre filters by exhaustive water elution. Dried filters were used for scintillation spectroscopy as previously described²⁶, yielding results comparable to trichloroacetic acid precipitation²⁶. Statistical analysis was by one-way ANOVA and Scheffe F -test. Asterisk, differs from control at $P < 0.01$. Two asterisks, differs from control at $P < 0.01$ and VIP at $P < 0.05$. c, To determine the labelling index, 40,000 cells were plated per 35 mm culture dish and a combination of tyrosine hydroxylase immunocytochemistry and autoradiography was used²⁶; the index is given by the proportion of tyrosine hydroxylase-positive cells that incorporate thymidine into the nucleus. Each value represents the mean for 3 dishes (300 cells from randomly selected, non-overlapping fields were scored on each dish) and is expressed as the mean $\% \pm$ s.e.m. Asterisk, differs from control at $P < 0.001$ by Student's t -test.

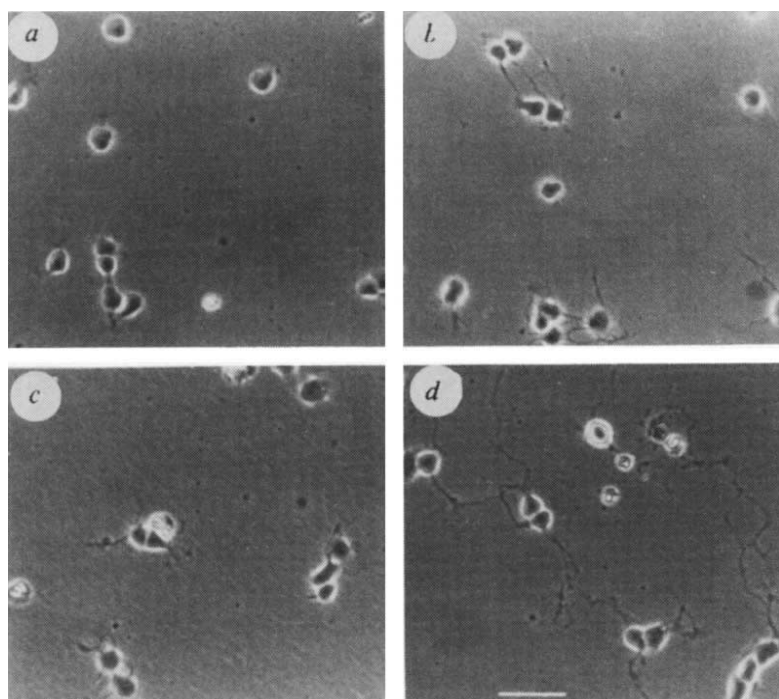


FIG. 2 Development of neuritic processes by embryonic neurons in culture. Cells were grown in control medium (a, c) or in medium containing 1 μM VIP (b, d). Living cultures were evaluated by phase contrast microscopy after 10 h (a, b) and 24 h (c, d). a and b, Ten hours after plating, neurite initiation was enhanced in the presence of VIP. c and d, After 24 h, the percentage of cells bearing long processes was increased by VIP. Scale bar, 50 μm .

METHODS. Neurons were cultured as in Fig. 1c in medium containing insulin (10 $\mu\text{g ml}^{-1}$), which is required for long-term survival *in vitro*⁵⁰.

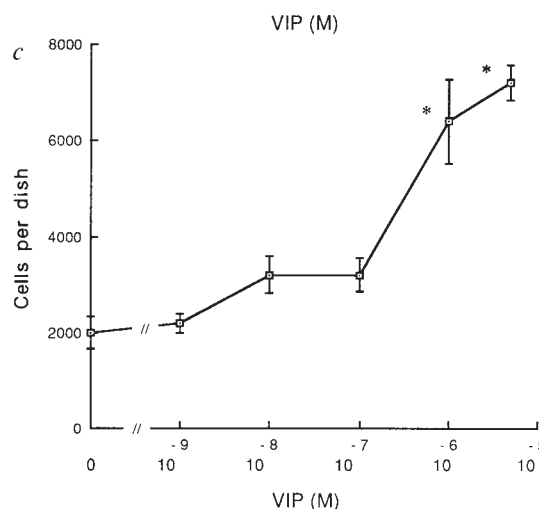
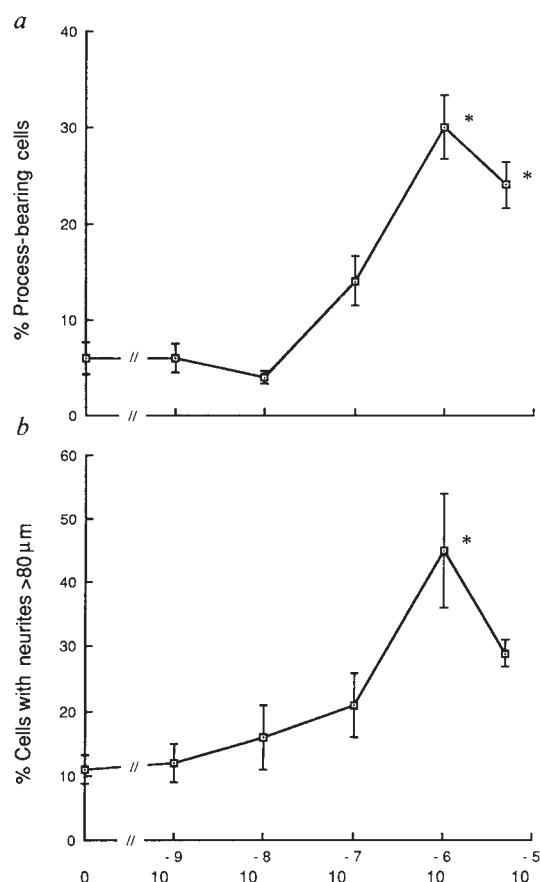


FIG. 3 Dose-response analysis of VIP effects on neurite outgrowth and neuronal cell number. Cells were cultured as in Fig. 2, either in control medium or in medium containing different concentrations of VIP. Cells were counted in living cultures using a phase contrast inverted microscope. *a*, Per cent of cells bearing processes 10 h after plating. *b*, Per cent of cells bearing long processes 24 h after plating. *c*, Neuronal cell number 4 days after plating.

METHODS. *a, b*, Neurite initiation was assessed 10 h after plating by determining the proportion of cells bearing a process >2 cell-diameters in length. Neurite elongation was assessed 24 h after plating by determining the percentage of cells bearing a process extending >80 μm from the cell body. Two hundred cells from randomly selected, non-overlapping fields were scored on each dish. *c*, For survival studies, additional peptide was added after 48 h. After 4 days *in vitro*, neurons in three randomly selected, non-overlapping 1 cm × 0.5 mm strips were counted (1.5% of the culture dish area). A neuron was defined as a round phase bright cell bearing a process extending >2 cell-diameters from the cell body. Data from triplicate cultures are expressed as the mean ± s.e.m. Statistical analysis was by one-way ANOVA and Scheffe *F*-test. *Differs from control at *P* < 0.01.

active in these experiments. Also, previous work in the adult SCG^{19,21,22}, as well as in adrenal chromaffin cells^{34,35}, has required VIP at micromolar concentrations to elicit effects. These findings suggest either a high peptidase activity in these cultured tissues, or the presence of a distinct class of receptor with a low affinity for VIP.

In summary, our observations indicate that a single neurotransmitter exerts diverse, unconventional, long-term actions on cultured neuroblasts. VIP stimulates mitosis, neurite outgrowth and survival. Our evidence indicates that the transmitter probably acts directly on the neuroblasts, and not through glial intermediaries, as has been proposed for central cultures^{36,37}. Finally, the presence of VIP in the embryonic SCG suggests a role for the peptide in the developing animal.

Neurotransmitters are known to regulate cell proliferation in several non-neuronal systems^{38,39}: in particular, VIP stimulates proliferation of cultured keratinocytes⁴⁰, smooth muscle cells⁴¹ and 3T3 cells⁴². Recently, acetylcholine analogues have been shown to stimulate DNA synthesis in cultures of brain glia⁴³.

Several lines of evidence suggest that our findings *in vitro* faithfully reflect *in vivo* mechanisms. VIP has been localized to presynaptic nerves that innervate neurons of the SCG, providing an anatomical substrate for these novel actions of the peptide^{19,24,25}. Moreover, as ganglion neurons are innervated as early as embryonic day 13 (ref. 44), trans-synaptic regulation could well occur at early stages. These observations indicate that VIP could be acting through conventional synaptic mechanisms to elicit unorthodox transmitter effects, but different routes also warrant consideration. VIP has been identified both in mature sympathetic neurons and in embryonic sympathetic

neuroblasts^{24,45-47}, so the peptide may act through autocrine⁴⁸ or even intracellular routes. Regardless of precise mechanisms, however, our results show that the scope of action of transmitter molecules may be far greater than formerly suspected. □

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TABLE 1 Specificity of InsP_3 -induced current in isolated vacuoles

Polyphosphoinositide(s)	Concentration (μM)	I (%) (mean $\pm$ s.d.)	N
Inositol 1,4-bisphosphate	20	6 ± 1	3
Inositol 1,3,4-trisphosphate	20	7 ± 2	3
Inositol 2,4,5-trisphosphate	20	15 ± 4	5
Inositol 1,3,4,5-tetrakisphosphate	20	4 ± 2	4
Inositol 1,3,4,5-tetrakisphosphate + inositol 1,4,5-trisphosphate	1	85 ± 5	3

The steady-state whole-vacuole current was first recorded for a vacuole clamped at +80 mV in an inositide-free medium. The vacuole was then washed first with medium containing selected inositide(s), and then with a medium containing 10^{-6} M InsP_3 . An inositide current was defined as the difference between the steady-state current with inositide and without inositide. Each inositide current I was normalized to that obtained with 10^{-6} M InsP_3 and multiplied by 100 to give I as a percentage. External and pipette media as described in Fig. 1 (5×10^{-3} M CaCl_2 in pipette medium). N , number of vacuoles tested.

Opening of Ca^{2+} channels in isolated red beet root vacuole membrane by inositol 1,4,5-trisphosphate

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INOSITOL 1,4,5-trisphosphate (InsP_3) is often implicated in pathways that couple extracellular signals to cellular calcium-dependent effectors. Various experiments have demonstrated that InsP_3 can release calcium from intracellular stores in animal cells¹⁻⁵ by acting on a specific receptor⁶. There are also reports that InsP_3 is important in plant cells⁷⁻¹². Using patch-clamp techniques¹³⁻¹⁵, we have now obtained direct measurements of a Ca^{2+} current in the isolated red beet root vacuole. This current was dependent on the InsP_3 concentration (Michaelis constant = 2.2×10^{-7} M at +80 mV) as well as the voltage across the vacuolar membrane. It was only partially inhibited by the calcium antagonists verapamil¹⁶ (2×10^{-5} M) and TMB8 (3,4,5-trimethoxybenzoic acid 8-diethylaminooctylester; 10^{-4} M)^{10,11}. Other inositides tested (inositol 1,4-bisphosphate; inositol 1,3,4- and 2,4,5-trisphosphates; inositol 1,3,4,5-tetrakisphosphate) did not produce a significant current even at concentrations up to 2×10^{-5} M. Single channels, opened by 10^{-6} M InsP_3 in isolated patches of the vacuole membrane, had conductances of 30 pS with 5×10^{-3} M Ca^{2+} in the vacuole and 10^{-3} M Ca^{2+} outside. They were voltage-dependent and opened only on depolarization of the vacuoles. Their open state showed extensive 'flickering', which did not depend on the Mg^{2+} concentration. These results show that InsP_3 releases calcium through an intact intracellular plant membrane by activating a Ca^{2+} channel, and that the opening of this channel is voltage-dependent.

Plant cells, like many other cells, maintain their cytoplasmic free Ca^{2+} concentration between 10^{-7} and 10^{-8} M (refs 17-19), probably by actively extruding Ca^{2+} from the cytoplasm towards the vacuole or out of the cell²⁰⁻²². The Ca^{2+} concentration in the vacuole (0.1-10 mM) is therefore higher than that in the cytoplasm¹⁷⁻¹⁹. All the experiments described here were performed using freshly isolated vacuoles of the red beet root in which the vacuolar currents at positive and negative voltages were blocked by an external medium containing high concentrations of unbuffered Ca^{2+} (10^{-3} M) and Zn^{2+} (10^{-4} M)^{23,24} (Fig. 1a). The current induced by InsP_3 was determined as explained in the legend to Fig. 1a.

The InsP_3 -induced current increased at positive potentials and was blocked at potentials more negative than -20 mV (Fig. 1b). The effects on this current of InsP_3 concentrations in the range 10^{-9} - 10^{-5} M were also studied (Fig. 2). In vacuoles held at +80 mV, the InsP_3 -induced current increased with increasing InsP_3 concentration. These data, fitted to a Michaelis-Menten equation gave an apparent Michaelis constant (K_m) of 2.2×10^{-7} M, indicating that there was no cooperativity as reported for animal cells²⁵. This value agrees with those found for plant vacuoles by other techniques (6×10^{-7} M for Oat¹⁰, and 2×10^{-7} M for Acer¹¹), but differs from that of 5.3×10^{-6} M reported for the vacuole of the fungus *Neurospora crassa*¹².

The polyphosphoinositides that were tested and their efficiency in activating a current are listed in Table 1. Only inositol 2,4,5-trisphosphate produced a current greater than 10% of the InsP_3 -induced current, even at 20 times the InsP_3 concentration. In this respect, inositol 2,4,5-trisphosphate has been previously shown to be more effective than the other InsP_3 derivatives³. Tetrakisphosphate (2×10^{-5} M) slightly inhibited the activation of channels by 10^{-6} M InsP_3 , as shown by applying tetrakisphosphate and InsP_3 together. This is not the case in animal cells^{3,26}.

With no calcium inside the vacuole, the outward current was not increased by InsP_3 , even at +80 mV. This indicated either that InsP_3 was unable to open channels in the absence of added vacuolar Ca^{2+} , or that InsP_3 opened channels, but neither K^+ or Cl^- , or both, could pass through them, regardless of the voltage (Fig. 1a). In other experiments, the effect of substituting Ca^{2+} with Ba^{2+} on the InsP_3 -induced current was examined. Vacuoles clamped at +80 mV in Ca^{2+} medium gave an average steady-state current of 412 ± 80 pA ($n = 29$; external and pipette media as described in Fig. 1; 5×10^{-3} M CaCl_2 in pipette medium; 10^{-6} M InsP_3 in the external medium). In another set of vacuoles with mole-for-mole replacement of CaCl_2 with BaCl_2 in both external and pipette solutions, the InsP_3 -induced current was 230 ± 70 pA ($n = 4$). Preliminary I - V curves indicate that there could also be a difference between the voltage sensitivities of the channel for these two ions (data not shown).

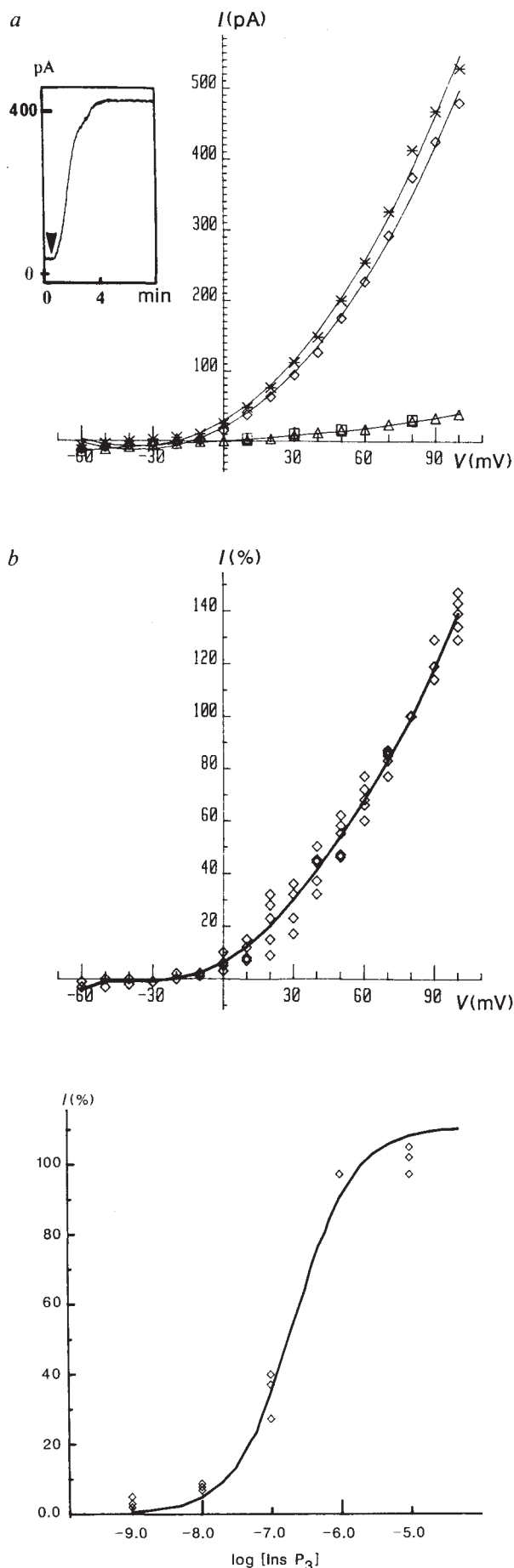
The effects of two Ca^{2+} antagonists on the InsP_3 -induced current were examined in the normal Ca^{2+} medium described above. Verapamil (2×10^{-5} M), a calcium-channel inhibitor¹⁶, decreased the InsP_3 -induced current by $66 \pm 5\%$ ($n = 3$). The addition of the antagonist TMB8 (10^{-4} M) to the external medium reduced the InsP_3 -induced current by $44 \pm 3\%$ ($n = 4$). These antagonists do not seem to specifically block the InsP_3 -induced current.

In the high Ca^{2+} plus Zn^{2+} external medium, almost none of the isolated patches showed voltage-gated channels for voltages from -100 to +100 mV. Of those patches that did not show

FIG. 1 The effect of InsP_3 on the tonoplast conductance: whole-vacuole I - V curves. External medium for all experiments: 0.6 M Sorbitol, 0.1 M KCl, 2×10^{-3} M MgCl_2 , 10^{-4} M ZnCl_2 , 5×10^{-3} M Tris-MES buffer, pH 7.5 (MES is 4, morpholine ethane sulphonic acid), 10^{-3} M CaCl_2 . Pipette medium: 0.6 M sorbitol, 0.1 M KCl, 2×10^{-3} M MgCl_2 , 5×10^{-3} M MES-Tris, pH 5.5, concentration of Ca^{2+} as indicated in each case. *a*, Example of the experiments to identify the InsP_3 -induced current in a vacuole. With 5×10^{-3} M Ca^{2+} in the pipette medium, control whole-vacuole currents (Δ) for 10-mV voltage steps from -60 to $+100$ mV were recorded 30 s after the step change in V . V was then held at $+80$ mV and perfusion with external medium containing 10^{-6} M InsP_3 was started at the time indicated by the arrow (inset). The perfusion was stopped when the current increased to a plateau (in 2 to 3 min), indicating that the medium in the chamber had been completely changed. Steady-state currents were again recorded for the same set of voltage steps as above ($\times$). InsP_3 was then washed out and the current was remeasured at $+80$ mV; data for vacuoles which had changed their properties during the experiment were not used for analysis. For each vacuole, the InsP_3 -induced current ($\diamond$) for a given potential was defined as the difference between the current with InsP_3 ($\times$) and without InsP_3 (Δ). The curves are least-squares fits to the data. Currents ($\square$) were obtained, for the same voltage range, in another vacuole where Ca^{2+} was omitted from the pipette medium and 10^{-6} M InsP_3 was present in the external medium. The perfusion rate into the chamber ($150 \mu\text{l}$) was 100 – $250 \mu\text{l min}^{-1}$, too slow to permit analysis of the rate of action of InsP_3 . *b*, Voltage dependence of the InsP_3 current. The currents at different voltages for five vacuoles were normalized to their mean current at $+80$ mV, 382 pA, to reduce the variability between vacuoles. I (%) was then found by multiplying the normalized values by 100. Curves were fit to the data by the least-squares method.

METHODS. The red beet root vacuoles used were prepared by mechanical slicing of the tissue³⁰ and washing the cut surface with the external medium. Individual vacuoles ($45 \pm 5 \mu\text{m}$) were selected by aspirating into a micro-manipulated (De Fonbrune) pipette, and transferred to the recording chamber filled with external medium. The patch pipettes were pulled from soft glass (microhaematocrit capillary 'blue tip', Lancer Sherwood Medical) with a two-stage puller (PP83, Narishige). After filling the pipette with the pipette medium, its tip was dipped for a few seconds in a 30% Silane (chlorotrimethylsilane), 70% carbon tetrachloride solution and air-dried. Giga seals were usually obtained without fire-polishing the tip of the pipette. A short voltage pulse (1 V, 100 ms) was sometimes necessary to disrupt the membrane under the patch pipette, allowing the transition between the 'vacuole-attached' and the 'whole-vacuole' configurations. Once in this configuration, the current was recorded only 5–10 min later, a time delay being necessary for the pipette medium to replace the vacuolar solution. I and V were measured with a patch-clamp amplifier (RK300; Biologic, Meylan, France) and low-pass filtered at 300 Hz with an eight-pole butterworth filter (Kemo Ltd, UK). Data were stored on a video cassette recorder (Sony SL T50) and digitized at 900 Hz for computer analysis (HP 98580). The current measurements were taken during steps in the vacuolar potential V . Current I from the external medium into the vacuole is negative. V was measured with reference to the external medium. InsP_3 , TMB8 and verapamil were obtained from Sigma, other inositides from Calbiochem.

FIG. 2 InsP_3 sensitivity of the InsP_3 -induced current. The steady-state whole-vacuole current was first recorded for a vacuole clamped at $+80$ mV in InsP_3 -free external medium. The vacuole was then washed, first with a medium containing a selected concentration of InsP_3 , and then with a medium containing 10^{-6} M InsP_3 . The steady-state currents were recorded for each medium including that for the return to InsP_3 -free medium at the end. I (%), the InsP_3 -induced current for the selected concentration, was obtained by normalizing the current at that concentration to the current at 10^{-6} M InsP_3 for each of the three vacuoles tested ($\diamond$). The continuous curve was drawn using a Michaelis-Menten equation with an apparent K_m of 2.2×10^{-7} M. External and pipette media as described in Fig. 1 (5×10^{-3} M CaCl_2 in pipette medium). $[\text{InsP}_3]$, Molar concentration of InsP_3 .



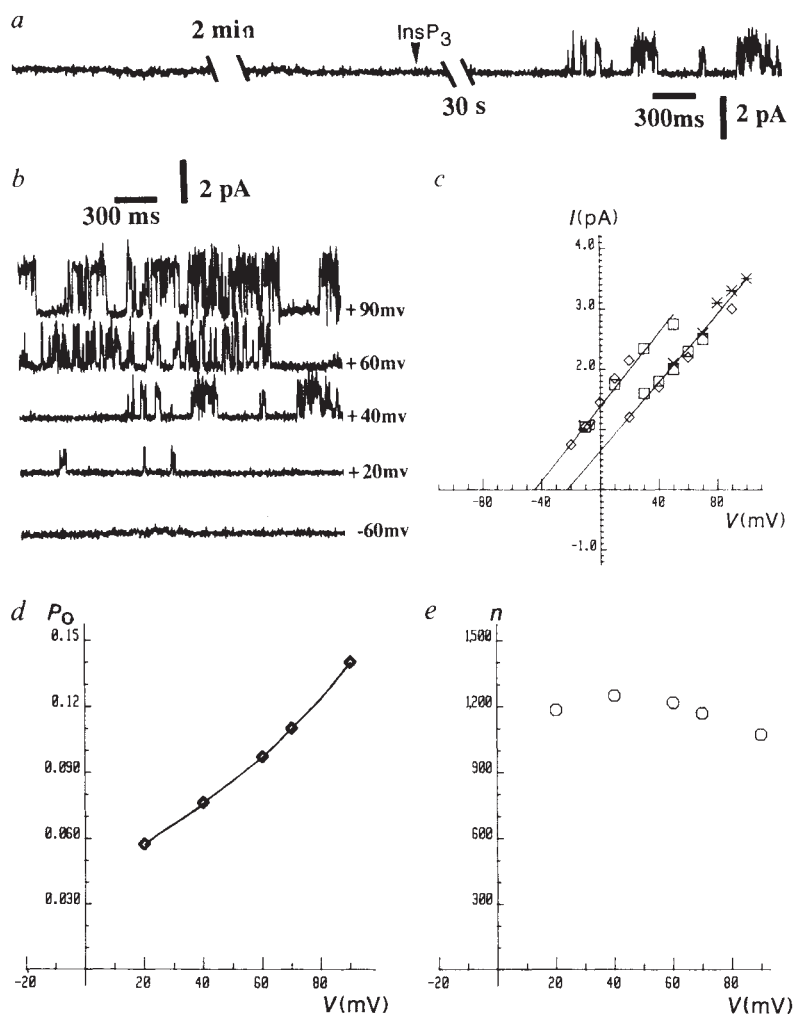


FIG. 3 Isolated InsP_3 -induced channels in outside-out patches. For all these experiments, the external medium was as described in Fig. 1. Concentrations of InsP_3 and Ca^{2+} as indicated in each case. Pipette medium as described in Fig. 1, with 5×10^{-3} M CaCl_2 . *a*, Current recorded in an outside-out patch at +40 mV. No channel openings occurred in the 2 min after patch formation and before 10^{-6} M InsP_3 was added at the time indicated by the arrow. InsP_3 arrival at the patch was delayed by ~ 30 s. *b*, Typical recordings of the channel currents at various pipette potentials. The closed state corresponds to the lower level in the recordings. The pipette potential was as indicated on each trace with 10^{-3} M CaCl_2 and 10^{-6} M InsP_3 in the external medium. *c*, I - V plot of single-channel currents from three patches ($\diamond$, $\square$, $\times$) at 10^{-3} M Ca^{2+} and 10^{-3} M InsP_3 or 10^{-4} M Ca^{2+} and 10^{-6} M InsP_3 in the external medium. Linear regression lines fitted to the data extrapolate to -22 mV and -44 mV in agreement with the Ca^{2+} gradients. The slope conductances for both were ~ 30 pS. Only data from patches showing one current level were used for these curves. *d*, Voltage dependence of channel opening probability P_o in an outside-out patch (10^{-3} M Ca^{2+} and 10^{-3} M InsP_3 in the external medium). P_o was calculated as the ratio of the open time to the total recording time of 2 min at different voltages. *e*, Number of InsP_3 -activated channels per vacuole, n , estimated as the ratio of the whole-vacuole current (in Fig. 1*a*) to the single-channel current with 10^{-3} M Ca^{2+} and 10^{-6} M InsP_3 in the external medium, multiplied by P_o at different voltages.

voltage-activated channels, $\sim 20\%$ had channels that were opened by 10^{-6} M InsP_3 . The results from such a channel in an outside-out patch are given in Fig. 3*a*. The channel opening was dependent on the vacuolar potential (Fig. 3*b*). Consistent with the whole-vacuole current recordings, there was no detectable inactivation of these channels even after up to 30 min. The open channels showed many flickering events which were not modified by removing Mg^{2+} ions from pipette and external solutions. From I - V plots of channel currents such as those shown in Fig. 3*c*, a slope conductance of 30 pS was calculated (Fig. 3*c*). These properties of the InsP_3 -activated channels were confirmed with vacuole-attached current measurements (10^{-6} M InsP_3 in the pipette).

Channel conductance remained nearly the same at 10^{-3} M or 10^{-4} M CaCl_2 in the external medium and with 5×10^{-3} M CaCl_2 in the pipette. For these two calcium gradients, the extrapolated reversal potentials corresponded to their calculated Nernst potentials (Fig. 3*c*). The channels were closed for many minutes at negative pipette potentials, precluding measurement of channel currents at these potentials. The open probability showed a marked positive voltage dependence (Fig. 3*d*) similar to that of the whole-vacuole InsP_3 -induced current (Fig. 1*b*). The number of channels, n , in the tonoplast, roughly estimated from the open probability and the whole-vacuole InsP_3 -induced current at given voltages, seemed to be independent of voltage (Fig. 3*e*). This could indicate tentatively that the voltage dependence of the whole-vacuole InsP_3 -induced current is due to the voltage dependence of the open probability for the single channel.

The InsP_3 - Ca^{2+} regulatory system, as well as its roles and mechanisms in plant cell physiology still remain to be fully elucidated^{9,27-29}. One possible role for the InsP_3 -activated

release of Ca^{2+} reported here is in a regulatory process in the tonoplast. In experiments performed with external calcium at 10^{-7} M, Ca^{2+} released by InsP_3 can close the nonspecific channels in the tonoplast as effectively as an increase in external Ca^{2+} . This effect depends on the Ca^{2+} buffer capacity outside the vacuole (J.A. *et al.*, manuscript in preparation).

The finding that the InsP_3 -activated channels remain closed at negative voltages and opened at positive voltages offers the interesting possibility of making calcium release rates independent of the kinetics of the InsP_3 synthesis pathway. Calcium release from intracellular stores can be primed by InsP_3 synthesis but achieved at the rate of the positive change in potential across the channel. □

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Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II

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THE macrophage scavenger receptor, which has been implicated in the pathogenesis of atherosclerosis^{1,2}, has an unusually broad binding specificity¹. Ligands include modified low-density lipoprotein and some polyanions (for example, poly(I) but not poly(C)). The scavenger receptor type I (ref. 3) has three principal extracellular domains that could participate in ligand binding: two fibrous coiled-coil domains (α -helical coiled-coil domain IV and collagen-like domain V), and the 110-amino-acid cysteine-rich C-terminal domain VI. We have cloned complementary DNAs encoding a second scavenger receptor which we have termed type II. This receptor is identical to the type I receptor, except that the cysteine-rich domain is replaced by a six-residue C terminus. Despite this truncation, the type II receptor mediates endocytosis of chemically modified low-density lipoprotein with high affinity and specificity, similar to that of the type I receptor. Therefore one or both of the extracellular fibrous domains are responsible for the unusual ligand-binding specificity of the receptor.

The C-terminal cysteine-rich domain of the type I receptor³ initially seemed to be an attractive candidate for the ligand-binding site. Similar domains are common features of membrane receptors, and a set of seven such domains defines the binding domain of the LDL (low-density lipoprotein) receptor⁴. We were therefore surprised to find a second group of cDNAs encoding a truncated scavenger receptor lacking this domain (type II receptor).

These cDNAs (Fig. 1) contain a 1,047-base pair (bp) open reading frame encoding a 349-amino acid protein with a calculated relative molecular mass of 38,388. Bases -25 to 1,042 (protein domains I-V, Fig. 2) of the type II scavenger receptor are identical to those in the type I receptor³, except for the synonymous replacement of T by C at position 750. Owing to the truncated C terminus, the α -helical coiled coil (domain IV) and collagen-like triple helix (domain V) comprise 86% of the extracellular portion of the type II receptor. The predicted secondary and quaternary structures (Fig. 2) are discussed in detail in the accompanying article³. Northern blot analysis (Fig.

3) using a type II receptor-specific probe shows that tissue-specific expression of the corresponding messenger RNA parallels that of the scavenger receptor (high expression in lung macrophages and whole lung, very low or undetectable expression in muscle and brain)^{3,5}. Studies are underway to determine whether the different receptors are derived from alternative splicing of a single gene.

To determine whether the type II receptor lacking the cysteine-rich domain could function as an endocytic receptor, we trans-

-35 taaatcggtgctgcgctctttaggacatgaagt -1	
1 ATGCCACAGTGGGACTTCTGATCAGCAGAGGACACTGACAGCTGTACAGAGTCT	60
1 M A Q W D D F P D Q Q E D T D S C T E S	20
61 GTGAAGTTTCGATGCTCGCTCAGTGACAGCTTGTGCTTCTCCCATCTTAAATGGCCCA	120
21 V K F D A R S V T A L L P P H P K N G P	40
121 ACTCTTCAAGAGAGGATGAAGTCTTATAAACTGCAGTATGACCTTTATCTCATGTTG	180
41 T L Q E R M K S Y K T A L I T L Y L I V	60
181 TTTGAGTTTCTCGTCCCATCATTGGCAGTGAGTGGCAGCTCAGCTCTGAAATGGGAACG	240
61 F V V L V P I I G I V A A Q L L K W E T	80
241 AAGAATTGACGGTTGGCTCAGTTAATGCAGATATATCTCCAAGTCCGAAGGCAAGGA	300
81 K (N C T) V G S V N A D I S P S P E G K G	100
301 AATGGCAGTGAAGATGAAATGAGATTTCGAGAAGCTGTGGAACGCATGAGCAACATG	360
101 (N G S) E D E M R F R E A V M E R M S N M	120
361 GAAAGCAGATCCAGTATCTTTCAGATAATGAAGCCAATCTCCTAGATGCTAAGAATTC	420
121 E S R I Q Y L S D N E A N L L D A K N F	140
421 CAAAATTCAGCATAACAACATGATCAAGATTTAATGATGTTCTTTCCAGCTAAATTC	480
141 Q (N F S) I T T D Q R F N D V L F T Q L N S S	160
481 TTACTTCTCCATCCAGGAACATGAGAATATCATAGGGGATATCTCCAAGTCATTAGTA	540
161 L L S S I Q E H E N I I G D I S K S L V	180
541 GGCTGTAACACCCAGTACTTGATTGTCAGTATGAAACACATGAATGGCAGAGTC	600
181 G L (N T T) V L D L Q F S I E T L N G R V	200
601 CAAGAGAATGCATTTAAACAACAGAGAGAGTCCGTAATAGAGGAGCGTATATACAAAT	660
201 Q E N A F K Q Q E E M R K L E E R I Y (R)	220
661 GCATCAGCAGAAATTAAGTCTTAGATGAAACCAAGTATTTGGAACAGGAAATAAA	720
221 A S A E I K S L L D E K V Y T L L E Q E I K	240
721 GGGAAATGAACATGTTGAATATATACCAATGATCTGAGGCTGAGGATTGGGAACAT	780
241 G E M K L L L N (N I T) N D L R L K D W E H	260
781 TCTCAGACATGAAATATCACTTTACTCCAGGTCCTCTGACCTCCAGGTCAGGTAATAA	840
261 S Q T L K (N I T) L L Q (G P P G P P G E K)	280
841 GGAGTAGAGGCCCTCTGGACAAATGTTATACCAAGCTTTCCAGGTCATTAAGTACT	900
281 (S D R L G P P G Q N G I P G F P G L I G T)	300
901 CCAGGTCTTAAGGTGATCGGGGATCTCTGTTTACCTGAGTTCAGGATTCAGGTA	960
301 P G L K L G D R G I S G L P G V R G F P I G	320
961 CCAATGGGGAAGACCGGGAAGCCAGGACTTAATGGCAAAAGGCCAGAGGGAGAAAAA	1020
321 P M G K T G K P G L N G Q K G Q K G E K	340
1021 GGGAGTGGACATGCAAAAGACCAAGTACCTGATGCTATTAGGCGAGGCCCTTT	1080
341 (S S G) S M Q R P G *	349

gaagatcaggtgggtgggggggaaccccgctgctaccatctcattaaaaggcccttcac
ctcggagcaagtcacagcagcagctgactcagaggtcccttggtggtcctccaaacag
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tcccagagaaattctatggttaacagatataatgagttcttaccctcaaacatata
gtctcagccagagatagtgatgcccataaaggaaatccactgtttgttcagcatatgtta
ccataacctcctggagctagacttaaacagctataaagatagctgtgtctctggccttc
cagaataattgtcctgaaacacatgactcaggaacacagcagtgatgcatgacagcag
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aaaaaaagtttccagatgtgttatgttactcaatgaataaataaaaaaaataaaataaa

FIG. 1 Nucleotide sequence of the bovine scavenger receptor type II from plasmids pBSR3 and pBSR9 (top), and alignment of the deduced amino-acid sequence (single-letter code, bottom). Isolation and sequencing of the clones were as described³. Bases are numbered 5' to 3', beginning with the first ATG. The putative transmembrane domain is underlined, potential N-linked glycosylation sites are enclosed by boxes with rounded corners, and collagen-like Gly-X-Y repeats are indicated by boxes with square corners. Plasmid pBSR3 (-35 to 2,004) contains 35 and 957 bp of 5' and 3' untranslated sequence, respectively. The 3' terminal 96 bases are derived from plasmid pBSR9 (isolated from an oligo(dT)-primed library), and include the polyadenylation signal (---) and a portion of the poly(A) tail. Analysis of independent clones has confirmed the sequence including the stop codon. The N-terminal 347 amino acids of the type I and type II scavenger receptors (including Cys 17 and Cys 83, highlighted in bold) are identical. The 110-amino acid C-terminal domain VI of the type I receptor, containing six cysteine residues, is replaced by a six-residue cysteine-free C terminus in the type II receptor.

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fected COS cells with a mammalian expression vector, plasmid pXSR3, and measured receptor activity (Fig. 4). Plasmid pXSR3 conferred high affinity and saturable receptor activity (Michaelis constant, K_m , $\sim 1 \mu\text{g}$ acetylated LDL protein per ml), which showed characteristic scavenger receptor specificity^{1,3}. Acetylated LDL, maleylated bovine serum albumin (BSA), poly(I) (Fig. 4b) and polyvinyl sulphate (data not shown), inhibited ¹²⁵I-labelled acetylated LDL internalization and degradation, whereas LDL, BSA and poly(C) did not. The type II receptor lacking the cysteine-rich domain can, therefore, mediate endocytosis of ¹²⁵I-labelled acetylated LDL, and its activity is remarkably similar to that of the type I receptor³.

The results described here indicate that one or both of the extracellular fibrous domains (IV and V) are crucial in determining the unusual specificity of the scavenger receptor. Collagenous domain V seems ideally suited for this role. The collagenous domains of several soluble proteins bind to a wide variety of molecules. Examples include asymmetric acetylcholine esterase binding to proteoglycans⁶, lung surfactant-associated apoproteins binding to lipids⁷, mannose-binding protein association with hydrophobic surfaces⁸, and, strikingly, the binding of complement factor C1q to DNA⁹. Collagen can bind lipids^{10,11} and many macromolecules¹², including fibronectin, fibrinogen, thrombospondin, laminin, and von Willebrand factor. A platelet secretory product or products with properties similar to those of von Willebrand factor can inhibit the binding and uptake of acetylated LDL by macrophages¹³. Indeed, LDL can bind to type I collagen, and this binding is enhanced by covalent modifications that convert LDL into a ligand of the scavenger receptor¹⁴. The predicted net positive charge of the collagenous domain (all 24 of the Gly-X-Y triplets are either uncharged or positively charged at neutral pH) could contribute to polyanionic ligand binding; but more subtle structural features are probably responsible for the specificity of polynucleotide binding (for example, binding of poly(I) but not poly(C)).

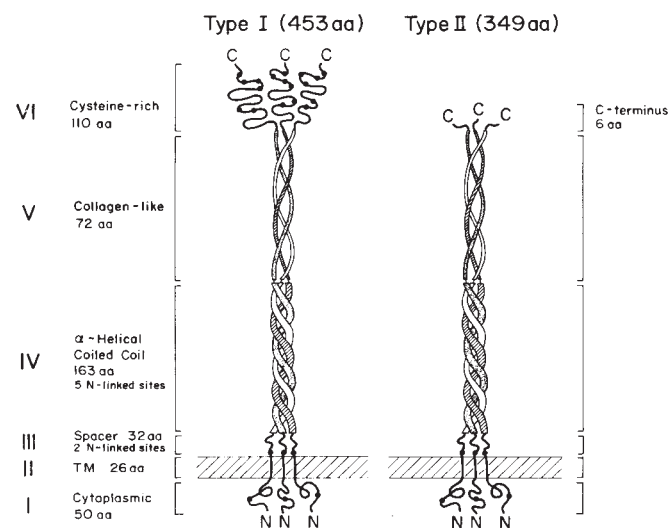


FIG. 2 Schematic model of the predicted structures of the type I and type II bovine scavenger receptors. The type I and type II receptors share five identical domains: I, N-terminal cytoplasmic (residues 1–50); II, transmembrane (residues 51–76); III, spacer (residues 77–108); IV, α -helical coiled coil (residues 109–271); and V, collagen-like (residues 272–343). The 110-amino-acid C-terminal cysteine-rich domain of the type I receptor is replaced by a six-residue C terminus in the type II receptor. Small filled circles in the cytoplasmic, spacer and cysteine-rich domains represent the approximate positions of cysteine residues. This model assumes that the α -helical coiled-coil domain IV forms a single triple-stranded left-handed superhelix that merges with the right-handed collagen-like triple helix of domain V to form a single long fibrous stalk. Alternative models based on higher-order oligomerization of the subunits are described in the accompanying article³. Abbreviations: aa, amino acids; TM, transmembrane.

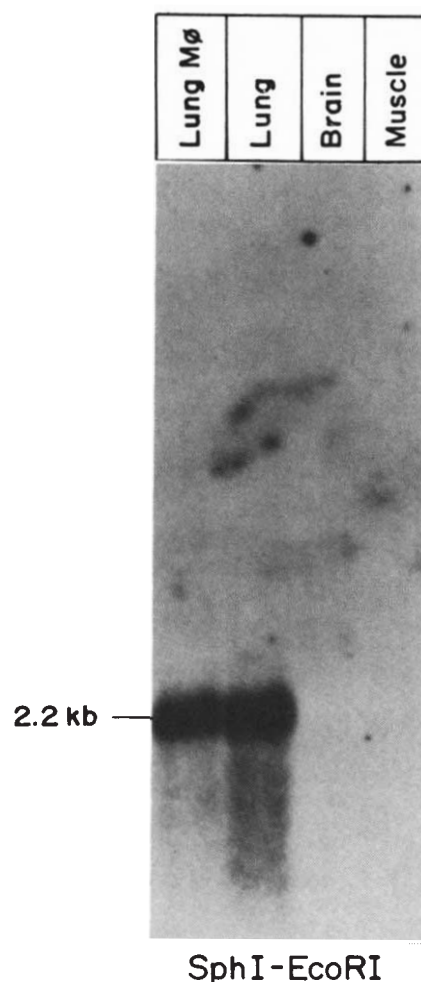


FIG. 3 Tissue-specific expression of scavenger receptor type II mRNA. Poly(A)⁺ RNAs from bovine alveolar macrophages, and bovine lung, brain and muscle tissue were isolated and subjected to RNA blotting as previously described³. A type II receptor-specific probe (*SphI-EcoRI* fragment, bases 1,234–2,004; Fig. 1) from plasmid pBSR3 was radiolabelled and used as the probe. Mφ, macrophage; kb, kilobases.

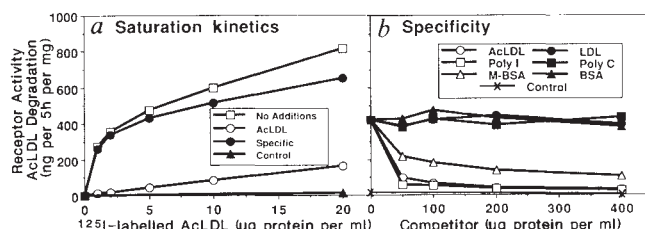


FIG. 4 Transient expression of high-affinity (a) and specific (b) scavenger receptor activity in COS M6 cells transfected with plasmid pXSR3. METHODS. An expression vector, plasmid pXSR3, was prepared by excision of the insert of plasmid pBSR3 by *Bam*H1 and *Xho*I, followed by ligation into plasmid pCDNA1 (Invitrogen)³. a, Indicated amounts of ¹²⁵I-labelled acetylated LDL in the absence (duplicate determinations, □) or presence (single determinations, ○) of 400 μg protein ml⁻¹ unlabelled acetylated LDL were added to the monolayers of plasmid pXSR3-transfected cells³, and receptor activity measured as the degradation of ¹²⁵I-labelled acetylated LDL³. Specific degradation was calculated by subtraction (●). Control values for the specific degradation of ¹²⁵I-labelled acetylated LDL by cells transfected with the vector without the cDNA insert (pCDNA1) were also determined (▲). b, In a separate experiment, the degradation of 5 μg protein ml⁻¹ ¹²⁵I-labelled acetylated LDL in the absence (triplicate determinations) or presence (duplicate determinations) of the indicated amounts of competitors was measured for cells transfected with plasmid pXSR3 or control plasmid pCDNA1 (X, acetylated LDL used as the competitor). Abbreviations: AcLDL, acetylated LDL; M-BSA, maleylated BSA.

Some of the specificity of the receptor could also be determined by its α -helical coiled-coil domain. The C-terminal portion of this domain (residues 204–271) seems to be a distinctive subdomain and is strikingly polar. Approximately 40% of the side chains are expected to be charged (25 Lys, Arg, Asp, Glu, His). The distribution of charges on the surface of the predicted superhelix³ could affect ligand specificity. In addition, post-translational modifications (including N-glycosylation^{3,5} and possibly hydroxylation of lysines and prolines, and glycosylation of hydroxylysines in the collagenous domain¹⁵) could also influence ligand binding as well as receptor assembly, stability, intracellular sorting and endocytosis.

The function of the C-terminal cysteine-rich domain of the type I receptor and the differences, if any, in the expression and physiological and pathophysiological functions of the type I and type II receptors are not known³. It is possible that coexpression of these two receptors results in assembly of mixed oligomers³. Several groups have reported unexpected differences in the cross-competition of acetylated LDL, oxidized LDL and maleyl BSA, and suggested that there are distinct scavenger receptor subtypes^{16–18}. Further studies of the expression and function of the type I and type II scavenger receptors should clarify these and other questions about these unusual receptors. □

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Stable expression of the bacterial *neo^r* gene in *Leishmania enriettii*

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MOLECULAR genetic studies in parasitic protozoa have been hindered by the lack of methods for the introduction and expression of modified or foreign genes in these organisms. Two recent reports described the transient expression of the bacterial chloramphenicol acetyl transferase (CAT) gene under the control of parasite-specific sequences^{1,2}. We now describe the stable expression of a selectable marker, the gene for neomycin resistance (*neo^r*) in *Leishmania enriettii*. A chimaeric gene containing the *neo^r* gene inserted between two α -tubulin intergenic sequences was introduced into the cells and drug-resistant *L. enriettii* were observed which stably expressed the *neo^r* gene. One goal of this work was to analyse the sequences necessary for *trans*-splicing of messenger RNA, as trypanosomatids have a novel process of RNA *trans*-splicing, described initially in *Trypanosoma brucei*^{3–5} and subsequently in several other trypanosomatids, including *L. enriettii*⁶. Many trypanosomatid genes are arranged in tandem arrays and the intergenic sequences contain both the splice acceptor site for the addition of the spliced leader sequence and a putative polyadenylation site⁶. Messenger RNA isolated from several different *neo^r* *L. enriettii* lines contained the spliced leader sequence joined to the *neo^r* gene at the position of the splice acceptor site in the α -tubulin intergenic sequence. The *neo^r* mRNA was also polyadenylated. Plasmid DNA is present within the drug-resistant organisms and appears to be extrachromosomal. The development of these methods allows the functional analysis of sequences necessary for *trans*-splicing.

Previous transient expression analysis using the pALT1-1 plasmid had shown that the α -tubulin intergenic sequence was required for expression of CAT¹. We therefore constructed a plasmid containing *neo^r* flanked by the intergenic regions of

the α -tubulin gene (Fig. 1a) for use in these experiments. pALT-Neo DNA was linearized by digestion with restriction endonuclease *SacI* and introduced into promastigote forms of *L. enriettii* by electroporation¹. After 6 days, resistant parasites were observed which grew in the presence of G418 (200 μ g ml⁻¹) whereas no growth was observed in mock-transfected cells. Figure 1b shows a growth curve of the resistant cells at various drug concentrations.

Messenger RNA from the drug-resistant parasites was analysed by northern blotting for the presence of *neo^r* mRNA (Fig. 2a). The results shown are representative of at least four independent transfections. The most abundant *neo^r* RNA species has a molecular size of 1.5 kilobases (kb) and is polyadenylated. Primer extension sequencing of the 5' end of the *neo^r* mRNA in the poly(A)⁺ fraction (Fig. 2b) shows that the spliced leader sequence has been added to the *neo^r* mRNA at the same acceptor site mapped for the α -tubulin mRNA⁶. The *neo^r* mRNA is processed (*trans*-spliced and polyadenylated) using the signals of the α -tubulin gene present in the pALT-Neo plasmid.

Drug-resistant parasites were screened for the presence of pALT-Neo DNA by Southern blotting. The major hybridizing bands (*neo^r* probe: 5.6 kb *EcoRI*, 0.79 kb *EcoRI/PstI*, 1.8 kb *XhoI*; pBluescript probe: 5.6 kb *EcoRI*, 4.8 kb *EcoRI/PstI*, 3.8 kb *XhoI*) correspond to the fragments expected to be produced by the digestion of either a circular pALT-Neo plasmid or a tandemly repeated form of the pALT-Neo plasmid. The results of a partial digestion of the DNA with *EcoRI* are consistent with a tandemly repeated structure (data not shown). In addition to these major fragments, there are several bands of lesser intensity: 9.4 and 1.8 kb bands after *EcoRI* digestion and a 5.6 kb band after *XhoI* digestion (Fig. 3a). These bands are consistent in both size and hybridization pattern with a model in which a subset of the tandem repeats have undergone recombination between the α -tubulin intergenic sequences, deleting either the *neo^r* gene or pBluescript sequences. Alternatively, the minor bands could represent an association of a subset of the pALT-Neo plasmids with cellular sequences. Cloning and sequence analysis are needed to confirm the identity of these bands.

Restriction enzyme analysis of the transfected DNA yielded an apparently circular restriction map. This suggests that the transfected DNA might exist as an extrachromosomal element⁷ or that the DNA could have integrated into the chromosome as

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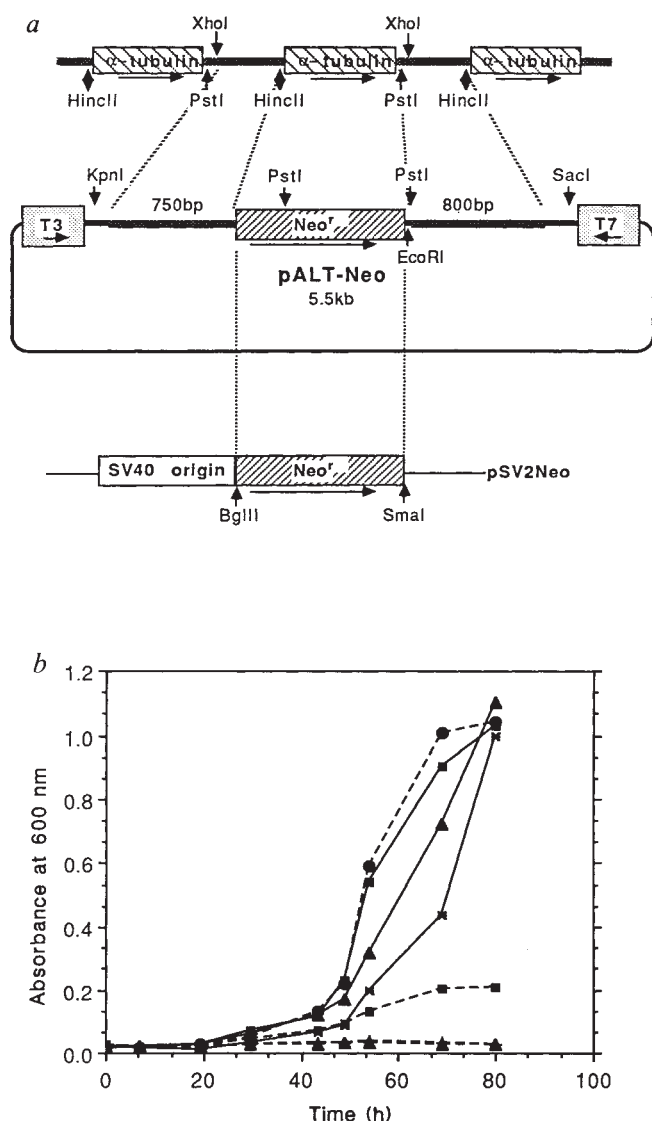


FIG. 1 Drug-resistant *L. enriettii* after transfection with pALT-Neo. **METHODS.** *a*, Plasmid pALT-Neo was derived in a stepwise cloning manner. The α -tubulin intergenic region was isolated from pLT1 (ref. 10) by digestion with *Xho*I and *Hinc*II and cloned into the *Xho*I and *Hinc*II restriction sites in the pBluescript vector (Stratagene). The *Hinc*II site is located five nucleotides 5' to the ATG codon for α -tubulin. The *Bgl*II/*Sma*I fragment containing the *neo*^r gene was isolated from pSV2Neo (ref. 13) after restriction digestion and cloned into the *Hinc*II/*Eco*RI sites of the vector after end-filling. Another copy of the α -tubulin intergenic region was isolated after *Pst*I/*Hinc*II digestion of pLT1 and cloned 3' to the *neo*^r gene in the *Pst*I/*Sma*I sites. *b*, Cells (5×10^7) from late log cultures were diluted 100-fold into fresh media containing different concentrations of G418; 0 $\mu\text{g ml}^{-1}$ (●), 30 $\mu\text{g ml}^{-1}$ (■), 100 $\mu\text{g ml}^{-1}$ (○), 200 $\mu\text{g ml}^{-1}$ (▲), and 2000 $\mu\text{g ml}^{-1}$ (*). Cell growth was monitored by measuring A_{600} . The dashed lines represent an untransfected culture and the solid lines a transfected line, *Le*₁(pALT-Neo). Cells were grown as previously described¹, except that drug-resistant cells were grown in the presence of G418 (200 $\mu\text{g ml}^{-1}$). Mid-log cultures (5×10^7 cells) of *L. enriettii* were transfected as previously described in the presence of 50 $\mu\text{g ml}^{-1}$ of linearized pALT-Neo, no DNA (mock-transfected) or pALT1-1(CAT)¹ (50 $\mu\text{g ml}^{-1}$), grown for 18–22 h in the absence of G418 and then selected in G418 (200 $\mu\text{g ml}^{-1}$). Growth was observed only in cells electroporated in the presence of pALT-Neo at a frequency of 10^{-7} – 10^{-6} . The frequency was estimated by counting the number of viable cells after 6 days. Control transfection experiments performed in the absence of DNA or in the presence of pALT1-1 (ref. 1) (CAT) DNA did not give rise to G418-resistant cells. From these experiments, we estimate that the rate of spontaneous G418 resistance must be lower than 5×10^{-7} . These experiments have been reproduced several times with identical results. The parasites are known to represent stable transfectants as they have been grown in the presence of G418 for at least 450 generations.

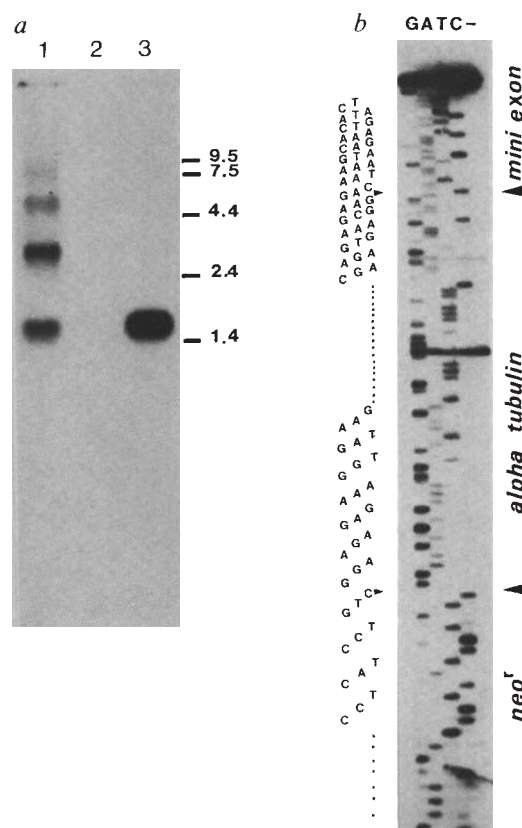


FIG. 2 The *neo*^r mRNA is polyadenylated and *trans*-spliced. **METHODS.** *a*, Total RNA (lane 1, 4 μg) was extracted from drug-resistant cells *Le*₁(pALT-Neo), fractionated into poly(A)⁻ (lane 2, 4 μg) and poly(A)⁺ (lane 3, 0.24 μg) and analysed by northern blot analysis with the *Pst*I fragment of the *neo*^r gene as a probe using previously described methods¹⁴. The size standards ($M_r \times 10^{-3}$) were visualized by staining the filter with methylene blue. There were several additional RNase sensitive (data not shown) bands which hybridized to the *neo*^r probe in the total RNA preparation. Their structure has not yet been determined. These bands were not recovered in either the poly(A)⁺ or poly(A)⁻ fractions, indicating that either the RNA is unstable under the conditions of fractionation or remains bound to the oligo(dT) column. *b*, Poly(A)⁺ RNA (2.4 μg) was analysed by primer extension sequencing⁶ using a radiolabelled *neo*^r-specific primer (CCGGAGAACCCTGCGTGCAATC) and analysed on a 6% acrylamide gel in 7M urea. The junctions between the spliced leader sequence and the α -tubulin intergenic region, and between the α -tubulin intergenic sequence and the *neo*^r sequence are marked with arrows.

a tandemly repeated structure⁸. If the pALT-Neo DNA had been incorporated into one of the *L. enriettii* chromosomes as a large tandem repeat a shift in the mobility of that chromosome when separated by pulsed field gel electrophoresis would be expected⁹. Under the conditions of this experiment, we observed no difference in the mobility of any of the bands when comparing non-transfected cells with transfected cells (Fig. 4a). In addition, there was no difference in the size of the α -tubulin-containing chromosome between the transfected and non-transfected cells (data not shown)¹⁰. When the *neo*^r probe was hybridized to the same gel, there was no hybridization to the chromosome containing the α -tubulin genes, indicating that the pALT-Neo DNA did not integrate into this locus. The *neo*^r probe did hybridize to some bands with an apparent size of 650–835 kb. A considerable amount of hybridization was seen at the top of the gel, and this may either represent nicked circles, as has been previously observed^{11,12}, or DNA associated with chromosomes⁸ which do not enter the gel under these conditions. The mobility of the 650–850 kb bands is extremely sensitive to low concentrations of ethidium bromide (data not shown), consistent with the model that these are circular molecules¹¹. By comparing the intensity of pALT-Neo bands to the α -tubulin bands in a Southern blot,

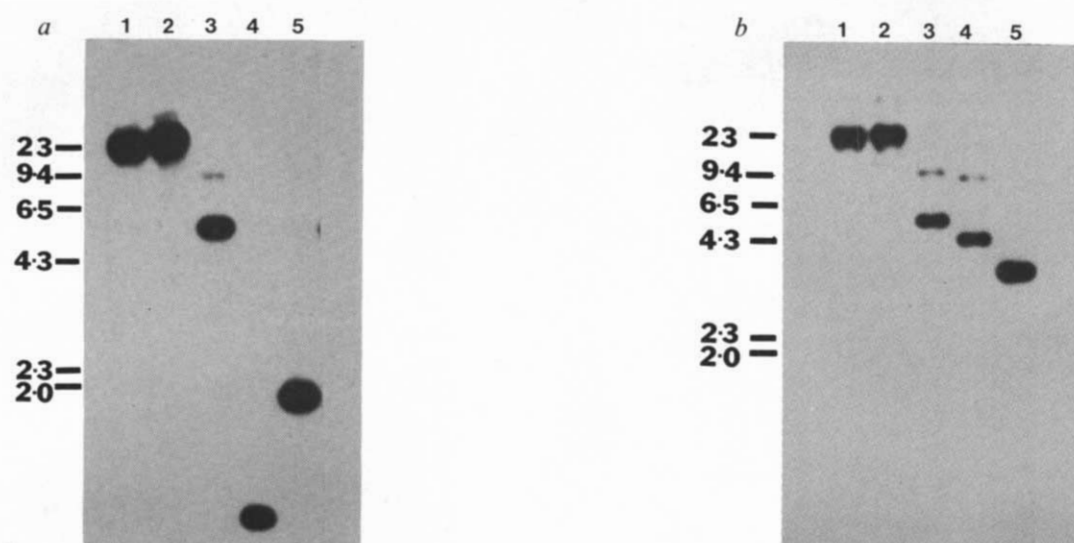


FIG. 3 pALT-Neo DNA is present in drug-resistant *L. enriettii*. DNA was isolated from G418-resistant cells, digested with restriction enzymes, resolved on a 0.8% agarose gel and analysed by probing parallel Southern blots with the *PstI neo'*-specific probe (a) or a pBluescript-specific probe (b).

Lane 1, undigested DNA; lane 2, *HindIII*-digested; lane 3, *EcoRI*-digested; lane 4, *EcoRI/PstI*-digested; lane 5, *XhoI*-digested. A similar Southern blot pattern is seen in DNA isolated from four independent transfections.

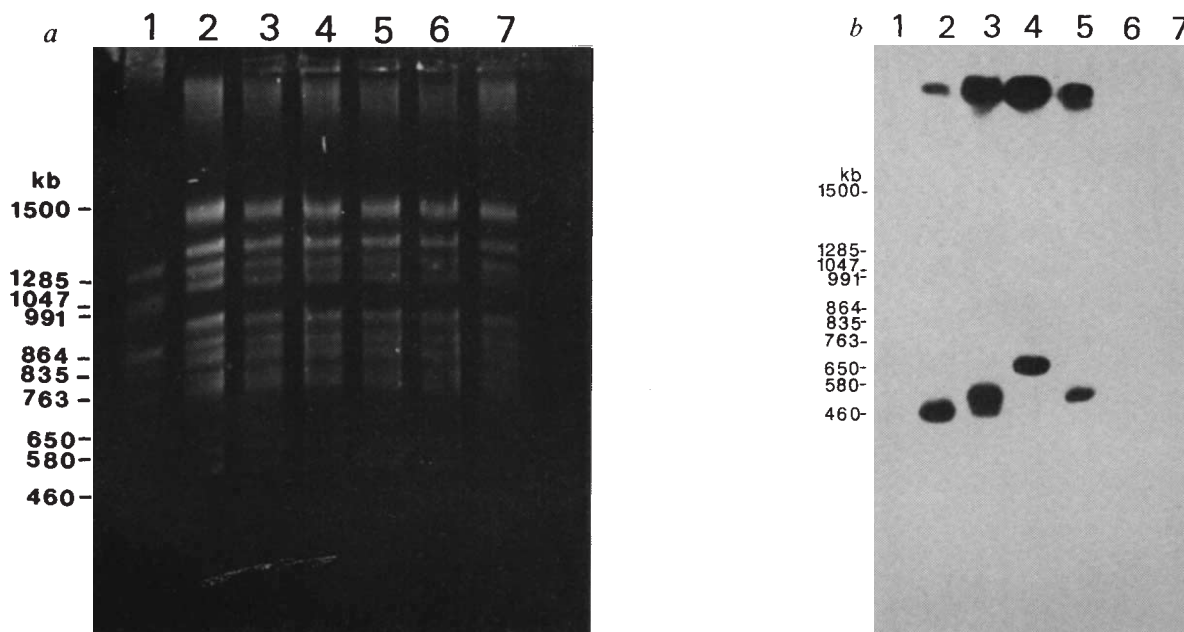


FIG. 4 pALT-Neo DNA is extrachromosomal. Size markers, $M_r \times 10^{-3}$. Transverse alternating pulsed field gel. Lane 1, yeast; lane 2, Le_1 (pALT-Neo); lane 3, Le_2 (pALT-Neo); lane 4, Le_3 (pALT-Neo); lane 5, Le_4 (pALT-Neo); lanes 6 and 7, control *L. enriettii*. a, An ethidium bromide-stained gel, b, an autoradiogram of a Southern blot probed with the *PstI* fragment specific for *neo'*. Hybridization with the pBluescript-specific probe gave the same pattern as the

neo'-specific probe (data not shown). Chromosomes, prepared as previously described⁹, of G418-resistant and control *L. enriettii* cells were analysed by transverse alternating pulsed field gel electrophoresis (Beckman) using buffer conditions suggested by the manufacturer (150 mA, 22 h, 1 min pulse time, 12 °C).

we estimate that each cell contains approximately 150 copies of the pALT-Neo DNA (data not shown).

We have described the stable expression of a selectable marker in *L. enriettii*. The *neo'* mRNA is processed (*trans*-spliced and polyadenylated) using the signals of the α -tubulin gene. The specific signals for transcription initiation remain to be determined. The pALT-Neo plasmid sequences are found to be stable in the drug-resistant parasites but the mechanism of propagation of these sequences also remains to be determined. □

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Active-site mutants altering the cooperativity of *E. coli* phosphofructokinase

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CRYSTAL structures of the high- and low-activity states of the allosteric enzyme phosphofructokinase implicate three arginines in substrate binding, catalysis and cooperativity¹⁻⁵. Arginines 162 and 243 reach into the active site from an adjacent subunit and interact with the cooperative substrate fructose 6-phosphate. Mutation of these arginines to serine results in mutant enzymes with reduced substrate binding and lowered cooperativity, but with little change in their catalytic ability (k_{cat}). Arg 72 bridges the two substrates fructose 6-phosphate and ATP, and interacts with the 1-phosphate of the product fructose 1,6-bisphosphate. Mutation of this residue to serine reduces the catalytic activity, cooperativity and binding of fructose 6-phosphate and fructose 1,6-bisphosphate. In the reverse reaction, the kinetics of wild-type and the Ser 72 mutant with respect to fructose 1,6-bisphosphate are hyperbolic, whereas those of the Ser 162 and Ser 243 mutants are sigmoidal. These results show that each of the three arginines contributes to cooperativity and to the transmission of allosteric signals between the four subunits of the enzyme.

We denote the Arg→Ser mutants by RS, followed by the mutant residue position. In the presence of 1 mM GDP, the wild-type and mutant phosphofructokinases (PFK) were allosterically activated and showed hyperbolic kinetics with respect to fructose 6-phosphate (Fru6P). But the Michaelis constant (K_m) for the binding of Fru6P of the mutants RS162, RS243 and RS72 were increased by 165-, 53- and 3-fold over wild-type, respectively (Table 1). Although these K_m values are not direct dissociation constants, the fact that the catalytic ability k_{cat} and $K_m[ATP]$ values for the mutants RS162 and RS243 are similar to those of wild type indicates that these mutations specifically reduce Fru6P binding. The relatively small increase in $K_m[Fru6P]$ for RS 72, coupled with its 33-fold decrease in catalytic activity, indicates that Arg 72 stabilizes the pentacoordinated transition state of the transferred phosphate relative to its four-coordinated ground state. This is consistent with the poor ordering of this residue in the crystal structure, indicating that it interacts only weakly with the substrates in the ground

state⁴. But the high K_m for the binding of fructose 1,6-bisphosphate (Fru1,6P₂) to this enzyme in the reverse reaction indicates that Arg 72 is particularly important for binding Fru1,6P₂.

In the absence of GDP, wild-type and mutant enzymes display cooperative kinetics with respect to Fru6P. The degree of cooperativity for the mutants, however, as measured by the Hill constant, is reduced from 4 to 2.1 (RS162), 2.7 (RS243) and 2.2 (RS72). Crystallographic¹⁻⁵ and kinetic⁶ studies indicate that the allosteric and cooperative behaviour of PFK is due to a reversible transition between two states that differ in affinities for Fru6P by a factor of at least 2,000. In the absence of Fru6P, the low-affinity T state predominates. Increasing concentrations of Fru6P increase the fraction of the high-affinity R state, leading to increased substrate binding and catalytic activity. In such a system, two features contribute to cooperativity; the ratio of the dissociation constants of the substrates in the R and T states, and the equilibrium constant $L=[R]/[T]$ in the absence of ligands⁷. Our kinetic data show that in the wild-type enzyme, Arg 162 contributes significantly to the binding of Fru6P in the R state. The absence of this residue in the T-state configuration⁵ (Fig. 1, left) would therefore lead to much lower Fru6P binding in the T state. Comparison between the T- and R-state structures indicates that in the absence of ligands, the T-state conformation with Gln 161 in the active site (Fig. 1, right) should be more stable than the R state because of repulsion of Arg 162 by the other active-site arginines. In the presence of Fru6P, however, the R-state conformation would be stabilized by the negative charge of the 6-phosphate. In addition, the Arg 72-Glu 241 salt bridge, which is absent in the R state, would also contribute to the increased stability of the T state. This model is consistent with the reduced cooperativity of the three mutants. Removal of any of the active-site arginines would either diminish the electrostatic repulsion or eliminate the stabilizing salt bridge. This would have the effect of decreasing L , resulting in the observed lower cooperativity of the mutants.

Allosteric activation by GDP is impaired for RS162: the half-saturation concentration of Fru6P in the presence of GDP ($K_m[Fru6P]$) is about the same as in its absence ($S_{1/2}$) instead of being much lower (Table 1), showing that for this mutant, GDP inhibits binding of Fru6P when it abolishes cooperativity.

In the reverse reaction, the kinetics of the wild-type and RS72 enzymes are hyperbolic with respect to Fru1,6P₂, owing to ADP acting both as a substrate and as an activator; unexpectedly, however, the kinetics of RS162 and RS243 are highly sigmoidal (Fig. 2). Their Hill coefficients are 2.0, and their $S_{1/2}[Fru1,6P_2]$ values ten times higher than those of the wild-type enzyme (Table 1). These results indicate that despite allosteric activation by ADP, the active sites of these mutants are still cooperatively linked. We suspect that maintenance of the salt bridge between

FIG. 1 Schematic view of the active site of R and T states of *E. coli* PFK. The active site of *E. coli* PFK is located in the interface between two adjacent subunits. In the absence of ligands, this interface is closed to Fru6P binding. In the presence of Fru6P, a series of quaternary and tertiary structural movements act in a concerted fashion to open the Fru6P-binding sites. Comparison of the R and T states of PFK from *Bacillus stearothermophilus*, which is closely related to the *E. coli* enzyme^{5,13}, demonstrates that one of the main rearrangements associated with the transition from the T to R state is a rearrangement of the end of helix 6 (loop 6-F)⁵. This results in the replacement of Glu 161 in the active site (Gln 161 in the *E. coli* enzyme) with Arg 162 and the severing of a salt bridge between Arg 72 and Glu 241 of the adjacent subunit. Asp 127 is the key catalytic residue in the reaction⁸. In the R state, the γ -phosphate is shown being transferred from ATP to Fru6P. About 3 Å separates the guanidinium groups of Arg 162 and Arg 243 from the 6-phosphoryl oxygens.

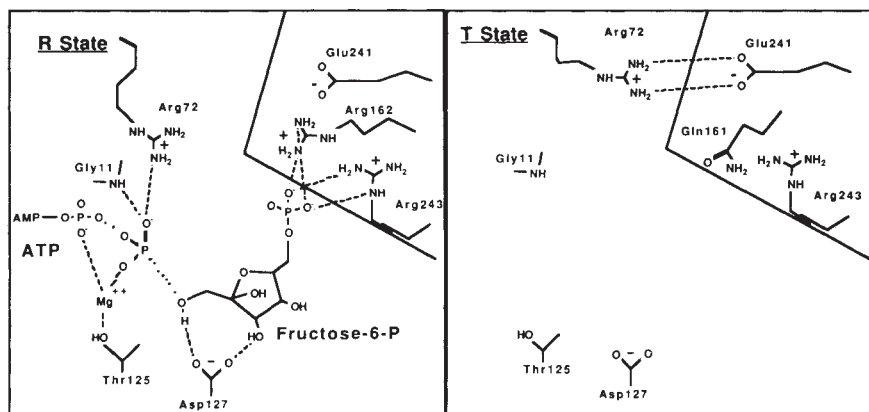


TABLE 1 Steady-state kinetic parameters of wild-type and mutant PFK

	Forward reaction					Reverse reaction			
	k_{cat} (s^{-1})	$K_{\text{m}}[\text{Fru6P}]$ (μM)	$K_{\text{m}}[\text{ATP}]$ (μM)	$S_{1/2}$ (μM)	n_{H}	k_{cat} (s^{-1})	$K_{\text{m}}[\text{ADP}]$ (μM)	$S_{1/2}[\text{Fru1,6P}_2]$ (mM)	n_{H}
Wild type	134	30	63	540	4	22	52	1.9	h
RS162	95	4,950	42	3,700	2.1	5	124	28.1	2.0
RS243	186	1,600	26	16,000	2.7	8.9	41	13.0	2.0
RS72	4	96	75	700	2.2	1.5	56	39.5	h

PFK catalyses the phosphorylation of Fru6P to Fru1,6P₂ by ATP. The enzyme from *Escherichia coli* (PFK-1; EC 2.7.1.11) is a tetramer and shows positive cooperativity with respect to Fru6P, allosteric activation by ADP or GDP, and allosteric inhibition by phosphoenolpyruvate (PEP). $S_{1/2}$, Fru6P concentration at half-maximal velocity in the absence of GDP; n_{H} , Hill constant determined from fitting kinetic data to $V = V_{\text{max}}(S_{1/2}^n + S^n)/S^n$; k_{cat} values were measured at saturating concentrations of both substrates and in the presence of 1 mM GDP. Catalysis in the wild-type enzyme is probably rate-limited by the phosphoryl transfer step⁸, although this has not been determined. $K_{\text{m}}[\text{Fru6P}]$ values were measured in the presence of 1 mM GDP. $K_{\text{m}}[\text{ATP}]$ values were obtained at saturating values of Fru6P. GDP was not included for these measurements as it acts as a competitive inhibitor for ATP. Reverse reaction kinetics for both wild type and RS72 are hyperbolic (h). Their $K_{\text{m}}[\text{Fru1,6P}_2]$ is equal to $S_{1/2}$. Arg-to-Ser mutations were chosen to eliminate the electrostatic interactions of these residues while still maintaining the polar character of the region. Mutants were produced using oligonucleotide-directed mutagenesis⁹. Wild-type and mutant enzymes were expressed from the plasmid pHL1¹⁰ in *E. coli* strain DF1020, which is deleted for both PFK genes¹¹. Enzymes were purified using a blue A column (Amicon)¹² and Sephacryl S-200 gel filtration chromatography (Pharmacia)⁸. In the forward direction, enzyme activity was measured at 25 °C, pH 8.0, in 100 mM Tris-HCl, 10 mM dithiothreitol, 10 mM MgCl₂, 10 mM NH₄Cl by coupling Fru1,6P₂ production to the oxidation of NADH⁸. Reverse reaction activity was measured as for the forward reaction, except the production of Fru6P was coupled to the reduction of NADP as described¹². Coupling enzymes and substrates were from Boehringer Mannheim. Kinetic parameters were obtained by fitting data to either Michaelis-Menten or Hill equations using the Enzfitter program (R. Leatherbarrow, personal communication). Measurements are the averages of at least three determinations.

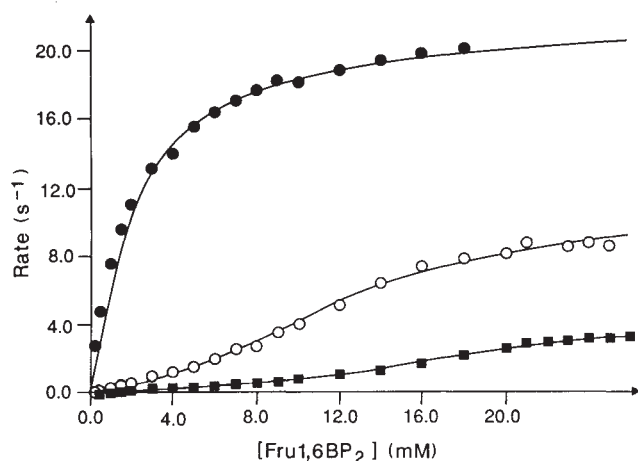


FIG. 2 Reverse reaction kinetics for wild-type, RS162 and RS243 enzymes. ●, Wild type; ○, RS243; ■, RS162. Curves represent best fits to Michaelis-Menten (wild type) or Hill (RS162 and RS243) equations, the parameters of which are given in Table 1.

Arg 72 and Glu 241 in the R state of these mutants may be responsible for this, but structural analysis will be required to verify this hypothesis.

In summary, electrostatic interactions between Arg 162, Arg 243 and the 6-phosphate of Fru6P bound to the neighbouring subunit stabilize the R state of PFK, whereas in the absence of Fru6P, a salt bridge between Arg 72 and Glu 241 of the neighbouring subunit stabilizes the T-state conformation. These

active-site arginines therefore play critical roles in the communication of cooperative and allosteric signals between subunits, particularly Arg 162, which performs the largest movement between the T- and R-state configurations. □

ERRATUM

Volatiles in submarine glasses as a discriminant of tectonic origin: application to the Troodos ophiolite

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Nature 343, 159–161 (1990)

THE figure legend to Fig. 1 of this letter was omitted and a legend from another paper used in error. The figure is shown below with the correct legend.

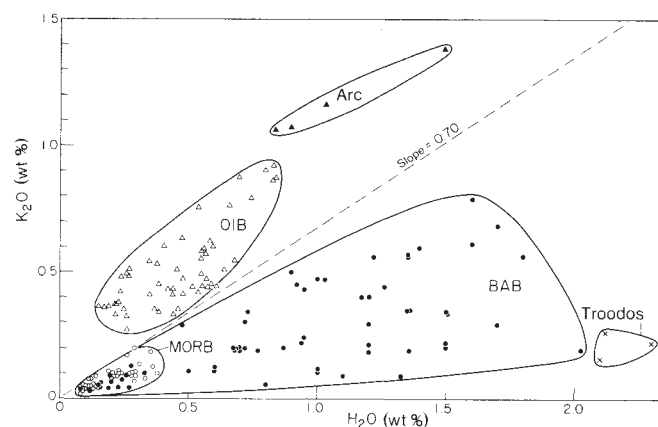


FIG. 1 K₂O plotted against H₂O for unaltered submarine volcanic glasses from known tectonic environments. MORB-field (○) (Mid-Atlantic Ridge¹³, East Pacific Rise¹⁴, Galapagos^{15,16}); BAB-field (●) (Mariana Trough¹², Scotia Sea¹⁷, Fiji/Lau¹⁸ and Woodlark basins¹⁹); OIB field (△) (Hawaii⁹⁻¹¹); Arc field (▲) (Mariana island arc¹²; Troodos (×) (this study). Note that MORB, BAB and Troodos fields have K₂O/H₂O < 0.70.

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